e-ISSN: 2459-1467

Online Türk Sağlık Bilimleri Dergisi

Online Turkish Journal of Health Sciences 2024;9(1):73-83

Online Türk Sağlık Bilimleri Dergisi 2024;9(1):73-83

## COX-mediated Regulation of Multiple Organ Damage by Betulin Treatment in Okadaic Acid-induced Alzheimer Rat Model

## Okadaik Asitle İndüklenen Alzheimer Sıçan Modelinde Betulin Tedavisi ile Çoklu Organ Hasarının COX Aracılığıyla Düzenlenmesi

<sup>1</sup>Ahmet Sarper BOZKURT, <sup>2</sup>Şenay Görücü YILMAZ

<sup>1</sup>Gaziantep University, Medicine Faculty, Physiology Department, Gaziantep, Türkiye <sup>2</sup>Gaziantep University, Health Science Faculty, Nutrition and Dietetics Department, Gaziantep, Türkiye

> Ahmet Sarper Bozkurt: https://orcid.org/0000-0002-7293-0974 Senay Görücü Yılmaz: https://orcid.org/0000-0003-0523-7819

#### ABSTRACT

**Objective:** Alzheimer's Disease (AD) is a progressive neurodegenerative disease. Cyclooxygenases (COXs) are essential in the inflammatory and regenerative processes of AD. This study aims to show that Betulin, a natural phytochemical (triterpene), is a candidate for COX-mediated correction of multiple organ damage of AD.

**Materials and Methods:** In this study, the effects and treatment potential of Betulin were investigated in the kidney, heart, and small intestine tissue in genetic, and histological contexts in an okadaic acid-induced rat AD model. A total of 36 Wistar albino male rats were included in the study. Cyclooxygenase 1 (COX-1) and Cyclooxygenase 2 (COX2) gene expressions were investigated by quantitative real-time PCR (qRT-PCR) in kidney, heart, and small intestine tissues. COX-1 and COX-2 proteins in tissues were analyzed by immunohistochemistry.

**Results:** COX-1 and COX-2 genes were detected to be overexpressed in the AD model. The expression of both genes was increased in the AD model and decreased after betulin treatment. Histological scores showed a strong positive effect of Betulin on the kidney, while it was relatively less effective on the heart and small intestine tissue. **Conclusions:** In treating organ damage in AD, COXs can be inhibited by Betulin and may be effective in functional recovery.

**Keywords:** Alzheimer's disease, Cyclooxygenase 1, Cyclooxygenase 2, betulin, organ damage

### ÖZ

Amaç: Alzheimer Hastalığı (AH) ilerleyici bir nörodejeneratif hastalıktır. Siklooksijenazlar (COX'ler), AH 'nin inflamatuar ve rejeneratif süreçlerinde gereklidir. Bu çalışma, doğal bir fitokimyasal (triterpen) olan Betulin'in, AH 'nin çoklu organ hasarının COX aracılı düzeltilmesi için aday olduğunu göstermeyi amaçlamaktadır.

**Materyal ve Metot:** Bu çalışmada okadaik asit ile indüklenen sıçan AH modelinde betulin'in böbrek, kalp ve ince bağırsak dokusundaki genetik ve histolojik bağlamdaki etkilerini ve tedavi potansiyeli araştırılmıştır. Çalışmaya 36 adet Wistar albino erkek sıçan dahil edildi. Böbrek, kalp ve ince bağırsak dokularında Cyclooxygenase 1 (COX1) ve Cyclooxygenase 2 (COX2) gen ekspresyonları kantitatif gerçek zamanlı PCR (qRT-PCR) ile araştırılmıştır. Dokulardaki COX-1 ve COX2 proteinleri immünohistokimya ile analiz edildi.

**Bulgular:** AH modelinde COX1 ve COX2 genlerinin aşırı eksprese edildiği tespit edildi. Her iki genin ekspresyonu AH modelinde artmış ve betulin tedavisinden sonra azalmıştır. Histolojik skorlar Betulin'in böbrek üzerinde güçlü bir olumlu etkisi olduğunu, kalp ve ince bağırsak dokusu üzerinde ise nispeten daha az etkili olduğunu gösterdi.

**Sonuç:** AH'de organ hasarının tedavisinde COX'lar betulin tarafından inhibe edilebilir ve fonksiyonel iyileşmede etkili olabilir.

Anahtar Kelimeler: Alzheimer hastalığı, Cyclooxygenase 1, Cyclooxygenase 2, betulin, organ hasarı

| Sorumlu Yazar / Corresponding Author:                            | Yavın Bilgisi / Article Info:              |
|--|--|
| Ahmet Sarper Bozkurt   | Gönderi Tarihi/ Received: 16/12/2023       |
| Department of Physiology, Gaziantep University, Medical Faculty, | Kabul Tarihi/ Accepted: 23/02/2024         |
| Gaziantep, Türkiye   | Online Yayın Tarihi/ Published: 11/03/2024 |
| Tel: +90-342 360 12 00/4707                                      |  |
| E-mail: asbozkurt@ganten.edu.tr                                  |  |

Attf / Cited: Bozkurt AS and Yılmaz ŞG. Cox-Mediated Regulation of Multiple Organ Damage by Betulin Treatment in Okadaic Acidinduced Alzheimer Rat Model. Online Türk Sağlık Bilimleri Dergisi 2024;9(1):73-83. doi: 10.26453/otjhs.1405878

## INTRODUCTION

Alzheimer's Disease (AD) is an increasingly common neurodegenerative disease that generally affects older people and impairs the quality of life of patients.<sup>1</sup> In today's world, with the aging population, there is also an increase in age-related, noncommunicable diseases such as dementia and its most common cause, Alzheimer's.<sup>2</sup> AD initially appears with memory loss and is accompanied by other cognitive functional impairments such as orientation difficulties and speech disorders. There are many questions about the etiopathogenesis of this disease. Betulin is a pentacyclic triterpene metabolite and is found in large amounts in the outer bark of birch trees (Betula, Betulaceae).<sup>4</sup> Recent studies conducted with Betulin also show that it has various properties useful in treating metabolic, cardiovascular, and neurological disorders.5 Betulin also reduces dietinduced obesity by inhibiting cholesterol and fatty acid biosynthesis. It also reduces the size of atherosclerotic plaques and increases their stability.6 COX-1 and COX-2 enzymes play a role in maintaining many physiological functions in living things, as well as appearing in pathological events.<sup>7</sup> COXs are expressed in at least two isoforms: COX1 is expressed in most tissues, while COX2 is primarily an inducible enzyme. COX2 expression increases rapidly in many tissues in response to tissue damage or the presence of proinflammatory cytokines.8 In the gastrointestinal tract, it has led to the idea that COX1 is critical for the cleansing action in the gastrointestinal mucosa. Therefore, COX2 appears to be responsible for inflammation.<sup>9</sup> In light of this information, determining the activity of two enzymes and possible treatment in case of multiorgan dysfunction may pave the way for systemic recovery.

In this study, the potential of Betulin to improve systemic organ dysfunctions and delay/prevent the occurrence of AD by regulating COX1 and COX2 enzymes that contribute to the inflammatory processes of AD was evaluated with genetic and immunohistochemical markers.

#### MATERIALS AND METHODS

*Ethics Committee Approval:* The study protocol was approved by Gaziantep University Animal Experiments Local Ethics Committee (Decision Number: 2023/29, Protocol Number: 323).

*Animals:* The animals were obtained from the Gaziantep University Experimental Animals Research and Application Center. All procedures respected the Guidelines of the European Union (86/609/EU). A total of 35 male Wistar Albino rats (8-12 weeks old, 250-300 gr) were included in the study.

#### **Experimental groups:**

1. Control Group (C) (n=6); The control group was

not treated.

2. DMSO Group (n=5): Dimethyl Sulfoxide (DMSO) is a Betulin solvent and was applied intraperitoneally (i.p.) once a day (between 9.00-11.00) for 4 weeks as 5 mg/ml (Thermo Fisher, D12345).

**3.** *Betulin Group (n=6):* The treatment agent Betulin (Selleckchem, Sylvanfield Drive, Houston, TX 77014 USA, Cat no. S4754) was dissolved in DMSO according to the commercial protocol. It was administered as 20 mg/kg/day i.p. every day for 4 weeks.<sup>1</sup>

**4.** *Phosphate Buffer Saline (PBS) Group (n=6):* The animals were placed in a stereotaxic chamber, and a total of 10  $\mu$ l of PBS was administered under anesthesia, 5  $\mu$ l to one side of the brain and 5  $\mu$ l to the other side.

5.Okadaic Acid (OKA) Group (n=6): Animals in this group were placed in the stereotaxic chamber under anesthesia. OKA was dissolved in DMSO according to the manufacturer's instructions. Animals received 5ml bilateral OKA once (200 ng/kg) (BioVision, Waltham, MA, USA, 78111-17-8in PBS<sup>2</sup> and were kept for 30 days for the AD model to form. According to the bregma coordinates (0.8mm posterior to bregma, 1.8mm lateral, and 3.6mm beneath the cortical surface), bilateral holes were drilled (OmniDrill35, 124 World Precision Instrument, Hertfordshire, UK) into the skull (ICV).

6. Okadaic Acid+Betulin Group (n=6): Animals were placed in the stereotaxic chamber under anesthesia. They received 5 ml bilateral OKA once (200 ng/kg) and were kept for 30 days for the model to form. At the end of the period, Betulin was administered once a day i.p.<sup>3</sup>

*Tissue Preparation:* The kidney, heart, and small intestine tissues of the animals were removed under xylazine + ketamine (5 mg/kg and 75 mg/kg) anesthesia, washed with PBS, divided into two, and stored in 10% formalin (for immunohistochemistry analyses) and RNA solution (for gene expression analyses).

Gene Expression Differences: Total RNA isolation from a 50 mg tissue sample was performed using the Triazole Method.<sup>10</sup> RNA concentration was meas-(260 nm) with a spectrophotometer ured (MultiSkan® Go, Thermo ScientificÔ), and samples were diluted (10 ng of RNA in 10 ml of complementary DNA (cDNA) reaction). Reverse Transcriptase PCR (RT-PCR) reactions were prepared according to the commercial kit (Abm Good, G236) protocol. The cDNA samples were incubated in a thermal cycler (Veriti, Thermo Fisher) (16°C, 30 minutes, 1 cycle; 42°C, 30 minutes, 1 cycle; 85°C, 5 minutes 1 cycle). At the end of the process, the products were immediately placed on ice and stored at -80°C until analysis. A 20 ml PCR reaction was prepared from

this cDNA library to analyze the expression changes of *COX-1* (Qiagen, QT00187859) and *s* (Qiagen, QT02486701) genes. The reaction contained 2X SYBR Green Reaction Mix (Qiagen), 10X Gene Expression Assay, and ddH<sub>2</sub>O. Expression data were normalized to the rat endogenous control beta-actin (ACTB) gene (Qiagen, QT00193473) and a universal rat reference RNA (Thermo Fisher, QS0641). The samples were incubated in the Real-Time PCR (Qiaegen, Rotor-Gene Q) device under two-step incubation conditions (95°C, 15 min, 1 cycle; 94°C-15 sec, 60°C-30 sec, 40 cycles). All measurements were analyzed in triplicate. The results were analyzed for DDCt. Fold change values (Fc) were calculated with  $2^{-\Delta\Delta Ct \ 11}$ 

Immunohistochemical Analysis: Tissues were removed from 10% formalin, dehydrated, embedded in paraffin, cooled, and 3 mm thick sections were cut. Samples were inhibited with endogenous peroxidase solution and then treated with antigen retrieval (Abcam, ab970). They were then kept in a normal blocking solution and blocked with avidin-biotin (Santa Cruz, sc-516217), incubated for 24 hours for primary antibody staining for COX-1 (Elabscience, E-AB-61656) and COX-2 (Elabscience, E-EL-R0792). In the next step, they were incubated with a biotin-labelled secondary antibody at the end of the incubation, and the samples were kept in streptavidin-HRP (Abcam ab7403). Finally, they were stained with a DAB Substrate Kit (Abcam, ab64238) and observed under a light microscope (Primo Star,

Zeiss). In evaluating COX-1 and COX-2 in each tissue, scoring was done based on the staining intensities and percentages of the stained area.<sup>12</sup>

Statistical Analysis: All analyses were performed in SPSS 22.0 (Release 22.0, SPSS Inc, Chicago, IL, USA). The normality of the groups was tested with Shapiro-Wilk. Tukey was used for post-hoc analyses, and One-Way ANOVA was used for the differences between the groups. The direction of significance was determined by descriptive statistics and multiple comparative analyses. Correlations were analyzed for the expression of the genes in each tissue. The Kruskal-Wallis Test was used to analyze the variances. p < 0.05 was considered statistically significant.

## RESULTS

In kidney tissue analysis, the difference between the groups was statistically significant for COX-1 (df (5.29), MS=204.294, F=31.612, P<0.05) and COX-2 (df (5.29), MS=133.187, F=28.218, P<0.05). Two genes were increased in AD rats, and they decreased after the Betulin treatment. For heart tissue, COX-1 (df (5.29), MS=163.943, F=55.585, P<0.05) and COX-2 (df (5.29), MS=164.509, F=123.060, P<0.05) expressions were found to be increased in AD and decreased in treatment. Small intestinal tissue was also statistically significant for COX-1 (df (5.29), MS=117.409, F=96.362, P<0.05) and COX-2 (df (5.29), MS=118.809, F=43.899, P<0.05) genes.

| Tissues | Genes | Experimental | n | Mean   | SD    | SE    | 95% CI for    | P-value |
|---------|-------|--------------|---|--------|-------|-------|---------------|---------|
|         |       | Groups       |   |        |       |       | Mean          |         |
| Kidney  | COX-1 | Control      | 6 | 37.787 | 2.066 | 0.843 | 35.619-39.954 | 0.001*  |
| -       |       | DMSO         | 5 | 36.288 | 3.864 | 1.728 | 31.490-41.086 |         |
|         |       | Betulin      | 6 | 22.888 | 0.307 | 0.126 | 22.566-23.211 |         |
|         |       | PBS          | 6 | 35.675 | 0.844 | 0.344 | 34.790-36.560 |         |
|         |       | OKA          | 6 | 37.907 | 4.040 | 1.650 | 33.666-42.147 |         |
|         |       | OKA+ Betulin | 6 | 37.510 | 2.034 | 0.830 | 35.375-39.645 |         |
|         | COX-2 | Control      | 6 | 38.283 | 1.116 | 0.456 | 37.112-39.455 | 0.001*  |
|         |       | DMSO         | 5 | 34.552 | 1.264 | 0.565 | 32.983-36.121 |         |
|         |       | Betulin      | 6 | 25.896 | 2.197 | 0.897 | 23.591-28.202 |         |
|         |       | PBS          | 6 | 35.452 | 3.859 | 1.575 | 31.402-39.501 |         |
|         |       | OKA          | 6 | 38.472 | 0.372 | 0.152 | 38.081-38.862 |         |
|         |       | OKA+ Betulin | 6 | 37.215 | 2.235 | 0.913 | 34.869-39.561 |         |
| Heart   | COX-1 | Control      | 6 | 39.467 | 0.326 | 0.133 | 39.124-39.809 | 0.001*  |
|         |       | DMSO         | 5 | 35.468 | 0.478 | 0.214 | 34.874-36.062 |         |
|         |       | Betulin      | 6 | 25.208 | 0.714 | 0.292 | 24.459-25.958 |         |
|         |       | PBS          | 6 | 36.580 | 2.788 | 1.138 | 33.654-39.506 |         |
|         |       | OKA          | 6 | 38.730 | 1.270 | 0.519 | 37.397-40.063 |         |
|         |       | OKA+ Betulin | 6 | 37.337 | 2.631 | 1.074 | 34.576-40.098 |         |
|         | COX-2 | Control      | 6 | 39.015 | 2.301 | 0.939 | 36.601-41.429 | 0.001*  |
|         |       | DMSO         | 5 | 33.232 | 0.337 | 0.151 | 32.814-33.650 |         |
|         |       | Betulin      | 6 | 24.510 | 0.365 | 0.149 | 24.127-24.893 |         |
|         |       | PBS          | 6 | 35.043 | 0.692 | 0.283 | 34.317-35.770 |         |
|         |       | OKA          | 6 | 37.462 | 0.339 | 0.138 | 37.106-37.817 |         |
|         |       | OKA+ Betulin | 6 | 37 057 | 1 282 | 0 523 | 35 712-38 402 |         |

Table 1. Descriptive statistics for COX-1 and COX-2 gene expressions in three tissues.

\*: P<0.05; SD: Standard deviation; SE: Standard error; CI: Confidence interval; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

|           | COX-1 | Control      | 6 | 35.352 | 0.921 | 0.376 | 34.385-36.319 | 0.001* |
|-----------|-------|--------------|---|--------|-------|-------|---------------|--------|
| Small     |       | DMSO         | 5 | 33.942 | 0.554 | 0.248 | 33.254-34.630 |        |
| Intestine |       | Betulin      | 6 | 25.140 | 0.720 | 0.294 | 24.385-25.895 |        |
|           |       | PBS          | 6 | 35.783 | 1.598 | 0.653 | 34.106-37.461 |        |
|           |       | OKA          | 6 | 35.468 | 0.298 | 0.121 | 35.156-35.781 |        |
|           |       | OKA+ Betulin | 6 | 37.505 | 1.905 | 0.778 | 35.506-39.504 |        |
|           | COX-2 | Control      | 6 | 35.683 | 1.365 | 0.557 | 34.251-37.116 | 0.001* |
|           |       | DMSO         | 5 | 32.938 | 0.511 | 0.228 | 32.304-33.572 |        |
|           |       | Betulin      | 6 | 25.110 | 1.080 | 0.441 | 23.977-26.243 |        |
|           |       | PBS          | 6 | 35.583 | 2.380 | 0.972 | 33.085-38.081 |        |
|           |       | OKA          | 6 | 36.387 | 0.979 | 0.400 | 35.359-37.415 |        |
|           |       | OKA+ Betulin | 6 | 36.875 | 2.416 | 0.986 | 34.340-39.410 |        |

Table 1. Continue.

\*: P<0.05; SD: Standard deviation; SE: Standard error; CI: Confidence interval; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Descriptive statistics for gene expressions in each tissue are given in Table 1.

Differences in group averages were tested with the Independent-samples Kruskal Wallis Test. Genes

were detected as significant for all 3 tissues (Figure 1).

Multiple comparative analysis of groups is given in Table 2.



Figure 1. The box-plot graph represents gene expression differences between the three tissues of group means. Comparative analyses were performed using the Independent-Samples Kruskal-Walli's test.

### Araştırma Makalesi (Research Article)

# Table 2. Multiple comparative analyses for groups in different tissues.

| Tissues | Genes | Experimental | Experimental             | Mean    | SE     | 95% CI for Mean              | P-value      |        |
|---------|-------|--------------|--------------------------|---------|--------|------------------------------|--------------|--------|
|         |       | Groups (1)   | DMSO                     | 1 /00   | 1 530  | 3 104 6 101                  | 0.022        |        |
|         |       |              | Betulin                  | 1/ 808  | 1.559  | 10.421 - 10.327              | 0.925        |        |
|         |       | Control      | DEtuini                  | 2 112   | 1.468  | 2 363 6 586                  | 0.001        |        |
|         |       | Control      | OV A                     | 0.120   | 1.400  | -2.303-0.380                 | 1.000        |        |
|         |       |              | OKA – Betulin            | -0.120  | 1.400  | 4 108 4 751                  | 1.000        |        |
|         |       |              | OKA+ Betuilli<br>Control | 1 400   | 1.400  | -4.190-4.731<br>6 101 2 104  | 1.000        |        |
|         |       |              | Dotulin                  | -1.499  | 1.539  | -0.191-3.194<br>9 707 18 002 | 0.923        |        |
|         |       | DMGO         | DDC                      | 0.612   | 1.539  | 0.707-18.093<br>4 080 5 206  | 0.001"       |        |
|         |       | DMSO         | F DS                     | 1 (10   | 1.539  | -4.080-5.500                 | 0.999        |        |
|         |       |              | OKA<br>OKA+ Datulin      | -1.019  | 1.539  | -0.311-3.074                 | 0.896        |        |
|         |       |              | OKA+ Detuilli            | -1.222  | 1.339  | -5.915-5.4/1                 | 0.900        |        |
|         |       |              | DMSO                     | -14.090 | 1.408  | 18.002 8.707                 | 0.001*       |        |
|         |       | D-4-1.       | DMSO                     | -13.400 | 1.339  | -18.0928.707                 | 0.001*       |        |
|         |       | Betulin      | PBS                      | -12./8/ | 1.408  | -1/.2018.312                 | 0.001*       |        |
|         |       |              | OKA   Datulia            | -13.018 | 1.408  | -19.49510.544                | 0.001*       |        |
|         | COX1  |              | OKA+ Detuilli<br>Control | -14.022 | 1.408  | -19.090-10.14/               | 0.001"       |        |
|         |       |              | Control                  | -2.111  | 1.408  | -0.380-2.303                 | 0.704        |        |
|         |       | DDC          | DMSO<br>Datalia          | -0.015  | 1.559  | -5.505-4.080                 | 0.999        |        |
|         |       | PBS          | Beluin                   | 12.787  | 1.408  | 8.512-17.201                 | 0.001^       |        |
|         |       |              | OKA                      | -2.232  | 1.468  | -6./06-2.24/                 | 0.654        |        |
|         |       |              | OKA+ Betulin             | -1.835  | 1.468  | -6.309-2.639                 | 0.809        |        |
|         |       |              | Control                  | 0.120   | 1.468  | -4.354-4.594                 | 1.000        |        |
|         |       |              | DMSO                     | 1.619   | 1.468  | -3.074-6.311                 | 0.896        |        |
|         |       | OKA          | Betulin                  | 15.018  | 1.468  | 10.544-19.492                | 0.001*       |        |
|         |       |              | PBS                      | 2.231   | 1.468  | -2.243-6.706                 | 0.654        |        |
|         |       |              | OKA+Betulin              | 0.397   | 1.468  | -4.078-4.871                 | 1.000        |        |
|         |       |              | Control                  | -0.277  | 1.468  | -4.751-4.197                 | 1.000        |        |
|         |       |              | DMSO                     | 1.222   | 1.539  | -3.471-5.914                 | 0.966        |        |
|         |       | OKA+Betulin  | Betulin                  | 14.622  | 1.468  | 10.147-19.095                | 0.001*       |        |
|         |       |              | PBS                      | 1.835   | 1.468  | -2.639-6.309                 | 0.809        |        |
|         |       |              | OKA                      | -0.397  | 1.468  | -4.871-4.077                 | 1.000        |        |
|         |       | Control      | DMSO                     | 3.731   | 1.315  | -0.279-7.741                 | 0.080        |        |
|         |       |              | Betulin                  | 12.387  | 1.254  | 8.563-416.210                | 0.001*       |        |
|         |       |              | PBS                      | 2.832   | 1.254  | -0.992-6.655                 | 0.955        |        |
|         |       |              | OKA                      | -0.188  | 1.254  | -4.012-3.635                 | 1.000        |        |
|         |       |              | OKA+ Betulin             | 1.068   | 1.254  | -2.755-4.892                 | 0.955        |        |
|         |       | DMSO         | Control                  | -3.761  | 1.315  | -7.741-0.279                 | 0.080        |        |
|         |       |              | Betulin                  | 8.655   | 1.315  | 4.645-12.666                 | 0.001*       |        |
|         |       |              | PBS                      | -0.899  | 1.315  | -4.910-3.111                 | 0.982        |        |
|         |       |              | OKA                      | -3.920  | 1.315  | -7.930-0.091                 | 0.058        |        |
|         |       |              | OKA+ Betulin             | -2.663  | 1.315  | -6.673-1.247                 | 0.354        |        |
|         |       | Betulin      | Control                  | -12.387 | 1.254  | -16.2118.563                 | 0.001*       |        |
|         |       |              |                          | DMSO    | -8.656 | 1.315                        | -12.6664.645 | 0.001* |
|         |       |              | PBS                      | -9.555  | 1.254  | -13.3795.731                 | 0.001*       |        |
| Kidney  |       |              | OKA                      | -12.575 | 1.254  | -16.3998.751                 | 0.001*       |        |
| Runey   | COVI  | PBS          | OKA+ Betulin             | -11.318 | 1.254  | -15.1437.495                 | 0.001*       |        |
|         | COX2  |              | Control                  | -2.832  | 1.254  | -6.655-0.992                 | 0.244        |        |
|         |       |              | DMSO                     | 0.900   | 1.315  | -3.111-4.910                 | 0.982        |        |
|         |       |              | Betulin                  | 9.555   | 1.254  | 5.731-13.379                 | 0.001*       |        |
|         |       |              | OKA                      | -3.020  | 1.254  | -6.846-0.803                 | 0.187        |        |
|         |       |              | OKA+ Betulin             | -1.763  | 1.254  | -5.587-2.060                 | 0.723        |        |
|         |       | OKA          | Control                  | 0.188   | 1.254  | -3.635-4.012                 | 1.000        |        |
|         |       |              | DMSO                     | 3.919   | 1.315  | -0.091-7.930                 | 0.058        |        |
|         |       |              | Betulin                  | 12.575  | 1.254  | 8.752-16.399                 | 0.001*       |        |
|         |       |              | PBS                      | 3.020   | 1.254  | -0.804-6.843                 | 0.187        |        |
|         |       |              | OKA+Betulin              | 1.257   | 1.254  | -2.567-5.080                 | 0.914        |        |
|         |       | OKA+Betulin  | Control                  | -1.068  | 1.254  | -4.892-2.755                 | 0.955        |        |
|         |       | STAT Dotumi  | DMSO                     | 2.663   | 1.315  | -1.347-6.673                 | 0.354        |        |
|         |       |              |                          | Betulin | 11.318 | 1.254                        | 7.495-15.142 | 0.001* |
|         |       |              | PBS                      | 7.763   | 1.254  | -2.060-5.587                 | 0.723        |        |
|         |       |              | OKA                      | -1.257  | 1.254  | -5.080-2.567                 | 0.914        |        |

\*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Table 2. Continue.

| Tissues | Genes | Experimental<br>Groups (I) | Experimental<br>Groups (II) | Mean            | SE     | 95% CI for Mean | P-value |
|---------|-------|----------------------------|-----------------------------|-----------------|--------|-----------------|---------|
|         |       | Control                    | DMSO                        | 3.400           | 1.040  | 0.828-1.469     | 0.007*  |
|         |       |                            | Betulin                     | 14.258          | 0.991  | 11.236-17.281   | 0.001*  |
|         |       |                            | PBS                         | 2.887           | 0.991  | -0.136-5.909    | 0.068   |
|         |       |                            | OKA                         | 0.737           | 0.991  | -2.286-3.759    | 0.975   |
|         |       |                            | OKA+ Betulin                | 2.130           | 0.991  | -0.893-5.153    | 0.292   |
|         |       | DMSO                       | Control                     | -3.999          | 1.039  | -7.1690:825     | 0.007*  |
|         |       |                            | Betulin                     | 10.260          | 1.039  | 7.089-13.430    | 0.001*  |
|         |       |                            | PBS                         | -1.112          | 1.039  | -4.282-2.058    | 0.889   |
|         |       |                            | OKA                         | -3.262          | 1.039  | -6.4320.092     | 0.041*  |
|         |       |                            | OKA+ Betulin                | -1.869          | 1.039  | -5.039-1.301    | 0.483   |
|         |       | Betulin                    | Control                     | -14.258         | 0.991  | -17.28111.236   | 0.001*  |
|         |       |                            | DMSO                        | -10.260         | 1.039  | -13.4307.089    | 0.001*  |
|         |       |                            | PBS                         | -11.372         | 0.991  | -14.3948.349    | 0.001*  |
|         |       |                            | OKA                         | -13.522         | 0.991  | -16.54410.499   | 0.001*  |
|         | COX1  |                            | OKA+ Betulin                | -12.128         | 0.991  | -15.1519.106    | 0.001*  |
|         | COM   | PBS                        | Control                     | -2.887          | 0.991  | -5.909-0.136    | 0.068   |
|         |       |                            | DMSO                        | 1.112           | 1.040  | -2.059-4.282    | 0.899   |
|         |       |                            | Betulin                     | 11.372          | 0.991  | 8.349-14.394    | 0.001*  |
|         |       |                            | OKA                         | -2.150          | 0.991  | -5.173-0.873    | 0.283   |
|         |       |                            | OKA+ Betulin                | -0.757          | 0.991  | -3.779-2.266    | 0.972   |
|         |       | OKA                        | Control                     | -0.737          | 0.991  | -3.759-2.286    | 0.975   |
|         |       |                            | DMSO                        | 3.262           | 1.040  | -0.872-5.172    | 0.041*  |
|         |       |                            | Betulin                     | 13.523          | 0.991  | 10.499-16.5442  | 0.001*  |
|         |       |                            | PBS                         | 2.150           | 0.991  | -0.873-5.173    | 0.283   |
|         |       |                            | OKA+Betulin                 | 1.393           | 0.991  | -1.629-4.416    | 0.724   |
|         |       | OKA+Betulin                | Control                     | -2.130          | 0.991  | -5.153-0.893    | 0.292   |
|         |       |                            | DMSO                        | 1.869           | 1.040  | -1.301-5.040    | 0.483   |
|         |       |                            | Betulin                     | 12.128          | 0.991  | 106-15.151      | 0.001*  |
|         |       |                            | PBS                         | 0.757           | 0.991  | -2.266-3.779    | 0.972   |
|         |       |                            | OKA                         | -1.393          | 0.991  | -4.416-1.629    | 0.724   |
|         |       | Control                    | DMSO                        | 5.783           | 0.700  | 3.649-7.917     | 0.001*  |
|         |       |                            | Betulin                     | 14.505          | 0.667  | 12.470-16.540   | 0.001*  |
|         |       |                            | PBS                         | 3.972           | 0.667  | 1.937-6.007     | 0.001*  |
|         |       |                            | OKA                         | 1.553           | 0.667  | -0.482-3.588    | 0.216   |
|         |       |                            | OKA+ Betulin                | 1.958           | 0.667  | -0.077-3.993    | 0.065   |
|         |       | DMSO                       | Control                     | -5.783          | 0.700  | -7.9173.649     | 0.001*  |
|         |       |                            | Betulin                     | 8.722           | 0.700  | 6.588-10.856    | 0.001*  |
|         |       |                            | PBS                         | -1.811          | 0.700  | -3.9462.095     | 0.001*  |
|         |       |                            | OKA                         | -4.230          | 0.700  | -5.9591.690     | 0.001*  |
|         |       | D . 1                      | OKA+ Betulin                | -3.825          | 0.700  | -5.959-1.690    | 0.001*  |
|         |       | Betulin                    | Control                     | -14.505         | 0.667  | -16.54012.470   | 0.001*  |
|         |       |                            | DMSO                        | -8.722          | 0.700  | -10.8566.588    | 0.001*  |
|         |       |                            | PBS                         | -10.533         | 0.667  | -12.5688.498    | 0.001*  |
|         |       |                            | OKA<br>OKA - D - 1          | -12.952         | 0.667  | -14.89/10.9/1   | 0.001*  |
| Heart   | COX2  | DDG                        | OKA+ Betulin                | -12.547         | 0.667  | -14.58210.512   | 0.001*  |
|         |       | PBS                        | Control                     | -3.972          | 0.337  | -6.00/1.93/     | 0.001*  |
|         |       |                            | DMSO                        | 1.811           | 0.700  | -0.323-3.946    | 0.133   |
|         |       |                            | Betulin                     | 10.533          | 0.667  | 8.498-12.568    | 0.001*  |
|         |       |                            |                             | -2.418          | 0.00/  | -4.4550.585     | 0.013*  |
|         |       | OVA                        | OKA+ Betulin                | -2.013          | 0.067  | -4.048-0.022    | 0.054   |
|         |       | UKA                        | Control                     | -1.555          | 0.00/  | -5.588-0.482    | 0.216   |
|         |       |                            | DMSU<br>Detrolin            | 4.230           | 0./00  | 2.095-6.364     | 0.001*  |
|         |       |                            | Betulin                     | 12.952          | 0.00/  | 10.91/-14.98/   | 0.001^  |
|         |       |                            | PBS                         | 2.418           | 0.00/0 | 0.383-4.433     | 0.013*  |
|         |       | OVALD (1                   | OKA+Betulin                 | 0.405           | 0.667  | -1.030-2.244    | 0.990   |
|         |       | OKA+Betulin                | Control                     | -1.958          | 0.00/  | -5.995-0.0/6    | 0.065   |
|         |       |                            | DMSU<br>Datu <sup>1</sup>   | 3.823<br>12.547 | 0.700  | 1.090-3.939     | 0.001   |
|         |       |                            | Beluiin                     | 12.34/          | 0.00/  | 10.312-14.382   | 0.001^  |
|         |       |                            | OKA                         | 2.015           | 0.007  | -0.022-4.040    | 0.034   |
|         |       |                            |                             | -0.705          | 0.007  | -2.770-1.030    | 0.770   |

\*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

Table 2. Continue.

| Control         DMSO         1.410         0.706         -0.743-3562         0.001*           Betulin         10.212         0.673         8.159-12.264         0.001*           PBS         -0.432         0.673         8.159-12.264         0.001*           OKA         -0.117         0.673         2.2484-1.620         0.987           OKA         -0.117         0.673         2.2484-1.620         0.987           OKA         -0.117         0.673         2.2462-0.131         0.001*           OKA         -1.410         0.706         -3.562-0.743         0.358           Betulin         8.802         0.706         -5.715-1.411         0.001*           OKA         -1.526         0.673         -12.2648.159         0.001*           PBS         -1.0423         0.673         -12.2648.159         0.001*           OKA         -10.212         0.673         -14.17-10.312         0.601*           OKA         -10.22         0.673         -14.20-2.483         0.897           PBS         -0.643         0.673         -12.306         0.001*           OKA         -10.212         0.673         -12.306         0.001*           DMSO         1   | Tissues   | Genes  | Experimental<br>Groups (I) | Experimental<br>Groups (II) | Mean    | SE    | 95% CI for Mean | P-value |
|--|-----------|--------|----------------------------|-----------------------------|---------|-------|-----------------|---------|
| *Snall         cox         Betulin         10.212         0.673         2.8484.1620         0.987           OKA         -0.117         0.673         -2.1639.1935         1.000           OKA         -0.117         0.673         -2.169-1935         1.000           OKA         -0.110         0.706         -3.562-0.743         0.368           Berulin         8.802         0.706         -3.562-0.743         0.368           OKA         -1.526         0.706         -3.679-0.625         0.226           OKA         -1.526         0.706         -3.579-0.625         0.226           OKA         -1.0212         0.673         -1.2245         0.601*           DMSO         -8.802         0.706         -10.521         0.601*           OKA         -10.328         0.673         -1.2495-8.531         0.001*           DMSO         1.841         0.706         -0.212.12.090         0.001*           PBS         -0.10232         0.673         -1.2491-4.630         0.001*           DMSO         1.841         0.706         -0.224.83         0.987           DMSO         1.841         0.706         -0.224.83         0.987           DMSO  |           |        | Control                    | DMSO                        | 1.410   | 0.706 | -0.743-3.562    | 0.368   |
| *Small         COX1         -0432         0.673         -2.484-1.620         0.987           OKA         -0.117         0.673         -2.169-1.935         1.000           OKA         Betulin         2.153         0.673         -4.2169-1.035         1.000           DMSO         Control         1.410         0.706         -5.562-0.743         0.368           Betulin         8.002         0.706         -5.362-0.743         0.368           OKA         -1.526         0.706         -5.3679-0.526         0.236           OKA         -1.520         0.706         -1.974-1.411         0.001*           OKA         -1.0312         0.673         -1.22648.159         0.001*           DMSO         -8.802         0.706         -1.0743         0.987           DMSO         -1.643         0.673         -1.240-2.433         0.0987           DMSO         1.841         0.673         -1.374-0.312         0.001*           OKA         Edulin         10.643         0.673         -1.374-0.330         0.141           OKA         Control         1.181         0.673         -1.377-0.371         0.997           OKA         Edulin         10.328         0.67  |           |        |                            | Betulin                     | 10.212  | 0.673 | 8.159-12.264    | 0.001*  |
| *Small         COXA         -0.117         0.673         -2.169-1.935         1.000           DMSO         Control         -1.410         0.705         -6.50-10.954         0.001*           Betulin         8.002         0.706         6.50-10.954         0.001*           OKA         -1.526         0.706         -5.679-0.626         0.2366           OKA         -1.526         0.706         -5.679-0.626         0.2366           OKA         -1.0212         0.673         -12.2448.159         0.001*           OKA         -10.212         0.673         -12.2648.159         0.001*           OKA         -10.423         0.673         -12.3808.276         0.001*           OKA         -10.432         0.673         -12.4958.591         0.001*           OKA         -10.432         0.673         -12.4058.591         0.001*           OKA         -10.432         0.673         -14.41710.312         0.001*           OKA         -10.432         0.673         -1.737-2.337         0.997           OKA         0.117         0.673         -1.737-2.337         0.997           OKA         0.315         0.673         -2.367-1.737         0.997   |           |        |                            | PBS                         | -0.432  | 0.673 | -2.484-1.620    | 0.987   |
| *Snall         COX         PBS         0.673         -4.205         0.010         0.035           Betulin         8.802         0.706         -5.562.0.733         0.001*           PBS         -1.841         0.706         -5.562.0.733         0.001*           OKA         -1.520         0.706         -5.362.0.734         0.001*           OKA         Heulin         -5.563         0.706         -5.715         -1411         0.001*           OKA         -0.010         -10.212         0.673         -12.264         -8.591         0.001*           DMSO         -8.802         0.673         -12.695         -8.591         0.001*           DMSO         -12.380         -8.276         0.001*         0.001*         0.001*           OKA         -10.328         0.673         -1.447         -10.312         0.673         -1.447         -10.312         0.001*           DMSO         1.841         0.763         -1.923         0.673         -1.2380         8.276         0.001*           DMSO         1.526         0.673         -1.722.0673         0.3774.0330         0.141           DMSO         1.526         0.763         -0.408+0.015         0.001*  |           |        |                            | OKA                         | -0.117  | 0.673 | -2.169-1.935    | 1.000   |
| *Small         CONTO         -1.410         0.706         -3.562-0.733         0.368           Betulin         8.802         0.706         -6.50-10.954         0.001*           PBS         -1.841         0.706         -3.679-0.626         0.286           OKA         Betulin         -0.573         -12.2448.159         0.001*           DMSO         -10.212         0.673         -12.2448.159         0.001*           OKA         -10.232         0.673         -12.2448.159         0.001*           OKA         -10.212         0.673         -12.3808.276         0.001*           OKA         -10.328         0.673         -12.3808.276         0.001*           OKA         -0.315         0.673         -1.620-2.483         0.987           DMSO         1.841         0.766         -0.620-3.671         0.127           Betulin         10.432         0.673         -1.737-2.036         0.141           OKA         0.315         0.673         8.276-12.380         0.001*           DMSO         1.526         0.763         4.26671         0.303.3774         0.300           OKA         Control         2.153         0.673         8.276-12.380 <td< td=""><td></td><td></td><td></td><td>OKA+ Betulin</td><td>-2.153</td><td>0.673</td><td>-4.205-0.101</td><td>0.035*</td></td<>   |           |        |                            | OKA+ Betulin                | -2.153  | 0.673 | -4.205-0.101    | 0.035*  |
| *Small         COX1         PBS         1.841         0.706         6.894-0.311         0.127           OKA         -1.526         0.706         -3.679-0.526         0.286           OKA         -1.526         0.706         -3.679-0.526         0.286           OKA         Feutin         3.563         0.706         -1.0214         0.001*           DMSO         -8.802         0.706         -10.9546.650         0.001*           OKA         10.328         0.673         -12.2648.591         0.001*           OKA         10.328         0.673         -12.3808.276         0.001*           OKA         Control         0.432         0.673         -1.738-0-8.276         0.001*           OKA         Control         0.432         0.673         -1.737-2.367         0.997           DMSO         1.841         0.763         -1.935-2.169         1.000           DMSO         1.526         0.706         -0.626-3.679         0.286           Betulin         10.328         0.673         -2.367-1.737         0.997           OKA         PBS         -0.315         0.673         -0.374-0.338         0.001*           PBS         1.722         0.673 <td></td> <td></td> <td>DMSO</td> <td>Control</td> <td>-1.410</td> <td>0.706</td> <td>-3.562-0.743</td> <td>0.368</td>   |           |        | DMSO                       | Control                     | -1.410  | 0.706 | -3.562-0.743    | 0.368   |
| *Small         COXA         -1.526         0.766         -3.679-0.026         0.286           OKA         -1.526         0.766         -3.679-0.026         0.286           OKA         10.212         0.673         -1.22648.159         0.001*           DMSO         -8.802         0.766         -10.2546.559         0.001*           PBS         -10.643         0.673         -12.3698.591         0.001*           OKA         -10.328         0.673         -12.4698.591         0.001*           OKA         -10.328         0.673         -12.4398.276         0.001*           OKA         -10.328         0.673         -12.4398.276         0.001*           OKA         -10.328         0.673         -12.411         0.127           Betulin         10.328         0.673         -1.273-2367         0.997           OKA         Eculin         10.328         0.673         -2.367-173.080         0.001*           DMSO         1.526         0.763         -3.774-0.30         0.997           OKA+Betulin         -2.0367         0.673         -2.367-173.80         0.001*           DMSO         3.563         0.773         -0.194.015         0.053  |           |        |                            | Betulin                     | 8.802   | 0.706 | 6.650-10.954    | 0.001*  |
| $ \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$  |           |        |                            | PBS                         | -1.841  | 0.706 | -3.994-0.311    | 0.127   |
| *Small         COX1         PBS         -0.73         -1.224-8.159         0.001*           PBS         -10.054         0.673         -1.224-8.159         0.001*           PBS         -10.054         0.673         -1.236-8.59         0.001*           OKA         -10.328         0.673         -1.236-8.59         0.001*           OKA         -10.328         0.673         -1.238-8.276         0.001*           OKA         -10.328         0.673         -1.4117-10.312         0.001*           OKA         -10.328         0.673         -1.4147-10.312         0.001*           OKA         -10.01         0.432         0.673         -1.4147-10.312         0.001*           OKA         -10.01         0.432         0.673         -1.372-3.67         0.997           OKA         -10.12         0.673         -1.372-3.67         0.997         0.673         -1.373-2.169         0.000           DMSO         1.526         0.763         -1.2380         0.001*         0.563         0.767         0.673         -2.367-1.737         0.997           OKA         PBS         -0.315         0.673         -0.30-3.774         0.141         0.5715         0.001*         0.563  |           |        |                            | OKA   Datalia               | -1.520  | 0.706 | -3.0/9-0.020    | 0.280   |
| *Small         COX1         DMSO         -8.802         0.703         -12.954         6.650         0.001*           PBS         -10.643         0.673         -12.954         6.650         0.001*           COX1         PBS         -10.643         0.673         -12.954         6.650         0.001*           COX1         PBS         -0.643         0.673         -14.172-10.312         0.001*           OKA         Betulin         10.643         0.673         -14.172-10.312         0.001*           OKA         0.315         0.673         -1.737-2.367         0.997           OKA         0.315         0.673         -1.737-2.367         0.997           OKA         Control         0.117         0.673         -2.367-1.737         0.001*           OKA         1526         0.706         -0.623-679         0.001*         0.001*           DMSO         1.525         0.673         -2.367-1.737         0.997         0.001*           OKA         PBS         -0.315         0.673         -0.303-374         0.001*           DMSO         3.563         0.706         1.4115-715         0.001*           DMSO         2.745         0.996         -2.995<   |           |        | Dotalin                    | OKA+ Beluin<br>Control      | -3.303  | 0.700 | -3./13-1.411    | 0.001*  |
| COX1         PBS<br>OKA         -10.043<br>-10.238         0.673<br>0.673         -12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-12.695<br>-1  |           |        | Detuini                    | DMSO                        | -10.212 | 0.075 | -10.954_6.650   | 0.001   |
| <ul> <li>*Small</li> <li>COX1</li> <li>PBS</li> <li>OKA</li> <li>-10.328</li> <li>0.673</li> <li>-12.380</li> <li>-8.276</li> <li>0.001*</li> <li>OKA</li> <li>-12.365</li> <li>0.673</li> <li>-14.417</li> <li>-10.312</li> <li>0.001*</li> <li>OKA</li> <li>-12.365</li> <li>0.673</li> <li>-1.620</li> <li>-1.737</li> <li>-2.367</li> <li>-1.737</li> <li>-1.737</li> <li>-1.737</li> <li>-1.737</li> <li>-1.737</li> <li>-1.740</li> <li>-1.7</li></ul>   |           |        |                            | PBS                         | -10.643 | 0.673 | -12 695-8 591   | 0.001*  |
| COX1         PBS         OKA+ Betulin         -12.365         0.673         -14.417         -10.312         0.001*           PBS         Control         0.432         0.673         -1.620-2483         0.987           Betulin         10.643         0.673         8.591-12.696         0.001*           Betulin         10.643         0.673         8.591-12.696         0.001*           OKA         0.315         0.673         -1.7137-2.367         0.997           OKA         0.315         0.673         -1.737-2.367         0.997           OKA         0.315         0.673         -1.737-2.367         0.997           OKA         0.315         0.673         -3.774-0.330         0.141           OKA         0.526         0.706         -0.626-3.679         0.286           Betulin         10.328         0.673         4.089-0.015         0.001*           DMSO         3.563         0.706         1.411-5.715         0.001*           DMSO         2.367         1.031-4.089         0.018         0.010*           PBS         1.722         0.673         -0.010+4.089         0.001*           DMSO         2.745         0.996         -0.291-5.782 <t< td=""><td></td><td></td><td></td><td>OKA</td><td>-10.328</td><td>0.673</td><td>-12.380-8.276</td><td>0.001*</td></t<>   |           |        |                            | OKA                         | -10.328 | 0.673 | -12.380-8.276   | 0.001*  |
| *Small<br>M*Small K*Small K*Smal   |           | 0.0714 |                            | OKA+ Betulin                | -12.365 | 0.673 | -14.417—10.312  | 0.001*  |
| *Small httsine COX2 PBS PS   |           | COXI   | PBS                        | Control                     | 0.432   | 0.673 | -1.620-2.483    | 0.987   |
| *Small<br>Intestine         COX2         Berulin<br>OKA         10.643<br>OKA         0.673<br>0.673         8.591-12.696<br>-1.737-2.367         0.001*<br>0.997           OKA         0.611         1.722         0.673         -3.774-0.330         0.141           OKA         Control         0.117         0.673         -3.774-0.330         0.141           OKA         Control         0.117         0.673         -3.774+0.330         0.014           PBS         0.315         0.673         8.276-12.380         0.001*           OKA+Betulin         10.328         0.673         8.276-12.380         0.001*           OKA+Betulin         2.0367         0.673         4.089.0015         0.033           OKA+Betulin         12.355         0.673         1.0312-14.417         0.001*           DMSO         3.563         0.706         1.411-5.715         0.001*           DMSO         2.745         0.996         -0.330-3.774         0.141           PBS         1.722         0.673         -0.015-4.089         0.033           Control         DMSO         2.745         0.996         -2.795-2.995         1.000           OKA         0.010         0.950         -2.782-0.291         0.975         0.674  |           |        |                            | DMSO                        | 1.841   | 0.706 | -0.311-3.994    | 0.127   |
| *Small Intestine COX2 PBS - Control 0.315 0.673 -1.737-2.367 0.997<br>OKA 0.315 0.673 -1.737-2.367 0.997<br>OKA+Betulin -1.722 0.673 -1.737-0.330 0.141<br>Control 0.117 0.673 -1.935-2.169 1.000<br>DMSO 1.526 0.706 -0.626-3.679 0.286<br>Betulin 10.328 0.673 8.276-1.2380 0.001*<br>PBS -0.315 0.673 -2.367-1.737 0.997<br>OKA+Betulin Control 2.153 0.673 -0.367-1.737 0.997<br>OKA+Betulin 12.365 0.673 0.101-4.205 0.001*<br>DMSO 3.563 0.706 1.411-5.715 0.001*<br>Betulin 12.365 0.673 -0.330-3.774 0.141<br>OKA 2.037 0.673 -0.291-5.782 0.094<br>Betulin 10.573 0.950 -2.795-2.995 1.000<br>OKA - 0.703 0.950 -2.795-2.995 1.000<br>OKA - 0.703 0.950 -3.599-2.192 0.0975<br>OKA+Betulin -1.192 0.950 -4.087-1.704 0.806<br>DMSO Control -2.745 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.485-0.485 0.019<br>PBS -2.645 0.996 -0.485-0.485 0.019<br>OKA +Betulin -3.937 0.996 -0.485-0.478 0.001*<br>PBS -10.473 0.950 -1.3.469-7.678 0.001*<br>PBS -10.473 0.950 -1.3.369-7.578 0.001*<br>DMSO -7.578 0.001*<br>DMSO 2.645 0.996 -0.391-5.682 0.019<br>OKA +Betulin -11.765 0.950 -1.4.660-8.869 0.001*<br>DMSO 2.645 0.996 -0.391-5.682 0.019<br>OKA +Betulin -11.765 0.950 -1.4.602-8.869 0.001*<br>DMSO 2.645 0.996 -0.313.69-7.578 0.001*<br>DMSO 2.645 0.996 -0.314-0.400 0.006*<br>Control 0.703 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.950 -7.402-4.85 0.019*<br>DMSO 3.937 0.950 0.712-6.485 0.019*<br>DMSO 3.937 0.950 0.712-6.485 0.019*<br>DMSO 3.937 0.950 0.724-2.487 0.001*<br>DMSO 3.937 0.950 0.734-2.407 0.995<br>OKA+Betulin 11.765 0.950 8.381-14.172 0.001*<br>DMSO 3.937 0.950 0.734-2.407 0.995<br>DMSO 3.937 0.950 0.704-0.877 0.806<br>DMSO 3.937   |           |        |                            | Betulin                     | 10.643  | 0.673 | 8.591-12.696    | 0.001*  |
| *Small COX2 PBS PBS -10.473 -3.774-0.330 0.141<br>OKA Control 0.117 0.673 -1.935-2.169 1.000<br>DMSO 1.526 0.706 -0.626-3.679 0.286<br>Betulin 10.328 0.673 2.367-1.737 0.997<br>OKA+Betulin -0.315 0.673 -2.367-1.737 0.997<br>OKA+Betulin -2.0367 0.673 -4.089-0.015 0.053<br>Control 2.153 0.673 0.101-4.205 0.001*<br>DMSO 3.563 0.706 1.411-5.715 0.001*<br>PBS 1.722 0.673 -0.330-3.774 0.141<br>OKA 2.037 0.673 -0.015-4.089 0.053<br>Control DMSO 2.745 0.996 -0.291-5.782 0.094<br>Betulin 10.573 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 -2.795-2.995 1.000<br>OKA+Betulin -1.192 0.950 -4.087-1.704 0.806<br>OKA -4Etulin -1.192 0.950 -4.085-0.391 0.001*<br>PBS -1.020 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.004<br>OKA - Betulin -7.820 0.996 -5.782-0.291 0.004<br>DMSO Control -1.192 0.950 -4.085 0.001*<br>PBS -1.0473 0.950 -7.678-13.469 0.001*<br>PBS -10.00 0.950 -2.795-2.995 1.000<br>OKA - Betulin -7.820 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.094<br>Betulin -7.820 0.996 -5.782-0.291 0.094<br>Betulin -7.820 0.996 -5.782-0.291 0.001*<br>PBS -10.473 0.950 -13.469-7.678 0.001*<br>PBS -10.473 0.950 -13.469-7.578 0.001*<br>DMSO -7.828 0.996 -0.391-5.788 0.001*<br>DMSO -7.828 0.996 -0.391-5.788 0.001*<br>DMSO -7.828 0.996 -0.391-5.682 0.019<br>OKA+ Betulin -1.277 0.950 -4.187-1.604 0.750<br>OKA+ Betulin -1.277 0.950 -4.187-1.604 0.750<br>OKA -Betulin -1.277 0.950 -4.187-1.604 0.750<br>OKA -Betulin -1.277 0.950 -4.187-1.604 0.750<br>OKA+Betulin -1.277 0.950 -4.187-1.604 0.750<br>OKA+Betulin -1.272 0.950 -3.384-2.407 0.995<br>OKA+Betulin -1.292 0.956 -3.582-0.991 0.956<br>OKA+Betulin -1.292 0.950 -3.384-2.407 0.995<br>OKA+Betulin -1.292 0.950 -3.384-2.407 0.995<br>OKA+Betulin -1.192 0.950 -3.384-2.407 0.995<br>OKA+Betulin -1.192 0.950 -3.384-2.407 0.995<br>OKA+Betulin -1.1765 0.950 -3.384-2.407 0.9956<br>OKA+Betulin -1.1765 0.950 -3.384-2.407 0.9956<br>OKA+Betulin -1.1765 0.950 -3.384-2.407 0.9956<br>OKA+Betulin -1.1765 0.950 -3.384-2.407 0.9956<br>OKA+Betulin -1.1765 0.950 -3.384-2.407 0.9956   |           |        |                            | OKA                         | 0.315   | 0.673 | -1.737-2.367    | 0.997   |
| <ul> <li>*Small Intestine</li> <li>COX2</li> <li>OKA</li> <li>Control</li> <li>0.117</li> <li>0.673</li> <li>1.935-2.169</li> <li>1.000</li> <li>DMSO</li> <li>1.526</li> <li>0.706</li> <li>-0.626-3.679</li> <li>0.286</li> <li>Betulin</li> <li>10.328</li> <li>0.673</li> <li>8.276-12.380</li> <li>0.001*</li> <li>PBS</li> <li>-0.315</li> <li>0.673</li> <li>4.089-0.015</li> <li>0.001*</li> <li>DMSO</li> <li>2.153</li> <li>0.673</li> <li>-0.101+2.025</li> <li>0.001*</li> <li>DMSO</li> <li>3.563</li> <li>0.706</li> <li>1.411-5.715</li> <li>0.001*</li> <li>DMSO</li> <li>2.745</li> <li>0.996</li> <li>-0.291-5.782</li> <li>0.094</li> <li>Betulin</li> <li>10.573</li> <li>0.950</li> <li>-2.795-2.995</li> <li>1.000</li> <li>OKA+ Betulin</li> <li>1.1272</li> <li>0.950</li> <li>-2.795-2.995</li> <li>1.000</li> <li>OKA + Betulin</li> <li>1.1273</li> <li>0.950</li> <li>-3.599-2.192</li> <li>0.975</li> <li>OKA + Betulin</li> <li>1.1273</li> <li>0.950</li> <li>-3.599-2.192</li> <li>0.094</li> <li>Betulin</li> <li>1.0573</li> <li>0.950</li> <li>-3.599-2.192</li> <li>0.094</li> <li>Betulin</li> <li>7.820</li> <li>0.996</li> <li>-5.682-0.391</li> <li>0.016</li> <li>006*</li> <li>0.095</li> <li>-2.795*2.095</li> <li>0.001*</li> <li>PBS</li> <li>-2.645</li> <li>0.996</li> <li>-5.682-0.391</li> <li>0.016*</li> <li>PBS</li> <li>-2.645</li> <li>0.996</li> <li>-5.682-0.391</li> <li>0.016*</li> <li>PBS</li> <li>-10.473</li> <li>0.950</li> <li>-13.469-7.678</li> <li>0.001*</li> <li>PBS</li> <li>-10.473</li> <li>0.950</li> <li>-13.69-7.578</li> <li>0.001*</li> <li>PBS</li> <li>-10.473</li> <li>0.950</li> <li>-14.670-7.840</li> <li>0.001*</li> <li>PBS</li></ul>   |           |        |                            | OKA+ Betulin                | -1.722  | 0.673 | -3.774-0.330    | 0.141   |
| *Small Intestine COX2 PBS Control DMSO 1.526 0.706 -0.626-3.679 0.286 Betulin 10.328 0.673 8.276-12.380 0.001* PBS -0.315 0.673 -2.367-1.737 0.997 OKA+Betulin 2-0367 0.673 -2.367-1.737 0.997 OKA+Betulin 2-0367 0.673 -4.089-0.015 0.053 0.001* DMSO 3.563 0.706 1.411-5.715 0.001* DMSO 3.563 0.706 1.411-5.715 0.001* DMSO 3.563 0.706 1.411-5.715 0.001* PBS 1.722 0.673 -0.30-3.774 0.141 OKA 2.037 0.673 -0.015-4.089 0.053 DMSO 2.745 0.996 -0.291-5.782 0.094 Betulin 10.573 0.950 7.678-13.469 0.001* PBS 0.100 0.950 -2.795-2.995 1.000 OKA -0.703 0.950 7.678-13.469 0.001* PBS 0.100 0.950 -2.795-2.995 1.000 OKA -0.703 0.950 -3.599-2.192 0.975 OKA+ Betulin 7.820 0.996 4.791-10.865 0.001* PBS -2.645 0.996 -5.682-0.391 0.116 OKA -3.449 0.996 -6.485-0.291 0.094 Betulin 7.820 0.996 4.791-10.865 0.001* PBS -2.645 0.996 -5.682-0.391 0.116 OKA -3.449 0.996 -6.485-0.485 0.019 OKA+ Betulin -7.820 0.996 -6.485-0.485 0.019 OKA+ Betulin -7.820 0.996 -6.485-0.485 0.019 OKA + Betulin -7.820 0.996 -6.485-0.485 0.019 OKA - 3.449 0.996 -6.485-0.485 0.019 OKA + Betulin -7.828 0.996 -10.865-4.791 0.001* DMSO -7.828 0.996 -10.865-4.791 0.001* DMSO 2.645 0.996 -0.391-5.682 0.116 OKA -11.277 0.950 -13.469-7.678 0.001* OKA -11.277 0.950 -14.60-8.869 0.001* OKA + Betulin -10.473 0.950 -7.578-13.369 0.001* OKA + Betulin -11.765 0.950 -7.578-13.369 0.001* OKA + Betulin -11.777 0.950 -7.41.871.604 0.750 DMSO 2.645 0.996 0.7381-3.692 0.975 DMSO 2.645 0.996 0.7381-3.682 0.116 OKA -0.003 0.950 -2.995-2.795 1.000 DMSO 2.645 0.996 0.7381-3.682 0.116 DMSO 2.645 0.996 0.7381-3.682 0.011* OKA -0.003 0.950 -3.381-14.172 0.950 -13.469-7.678 0.001* DMSO 2.645 0.996 0.741-2.6485 0.019* DMSO 2.645 0.996 0.7381-3.682 0.011* DMSO 2.645 0.996 0.7412-6.485 0.019* DMSO 2.645 0.996 0.7412-6.485 0.019* DMSO 2.645 0.996 0.7412-6.485 0.019* DMSO 3.3449 0.996 0.7412-6.485 0.019* DMSO 3.937 0.950 0.3381-14.172 0.901* DMSO 3.937  |           |        | OKA                        | Control                     | 0.117   | 0.673 | -1.935-2.169    | 1.000   |
| <ul> <li>*Small Intestine</li> <li>COX2</li> <li>*Small Intestine</li> <li>COX2</li> <li>PBS</li> <li< td=""><td></td><td></td><td></td><td>DMSO</td><td>1.526</td><td>0.706</td><td>-0.626-3.679</td><td>0.286</td></li<></ul>   |           |        |                            | DMSO                        | 1.526   | 0.706 | -0.626-3.679    | 0.286   |
| <ul> <li>*Small Intestine</li> <li>COX2</li> <li>PBS</li> <li>-0.315</li> <li>0.673</li> <li>-2.367-1.737</li> <li>0.997</li> <li>OKA+Betulin</li> <li>OKA+Betulin</li> <li>2.153</li> <li>0.673</li> <li>0.101-4.205</li> <li>0.001*</li> <li>DMSO</li> <li>3.563</li> <li>0.760</li> <li>1.411-5.715</li> <li>0.001*</li> <li>DMSO</li> <li>2.363</li> <li>0.673</li> <li>0.101-4.205</li> <li>0.001*</li> <li>DMSO</li> <li>3.563</li> <li>0.706</li> <li>1.411-5.715</li> <li>0.001*</li> <li>PBS</li> <li>1.722</li> <li>0.673</li> <li>-0.330-3.774</li> <li>0.141</li> <li>OKA</li> <li>2.037</li> <li>0.673</li> <li>-0.015-4.089</li> <li>0.001*</li> <li>PBS</li> <li>1.722</li> <li>0.673</li> <li>-0.30-3.774</li> <li>0.141</li> <li>OKA</li> <li>2.037</li> <li>0.673</li> <li>-0.30-3.774</li> <li>0.141</li> <li>OKA</li> <li>2.037</li> <li>0.673</li> <li>-0.015-4.089</li> <li>0.001*</li> <li>PBS</li> <li>0.100</li> <li>0.950</li> <li>-3.30-3.774</li> <li>0.141</li> <li>0.044</li> <li>Betulin</li> <li>10.573</li> <li>0.950</li> <li>-7.678-13.469</li> <li>0.001*</li> <li>PBS</li> <li>0.100</li> <li>0.950</li> <li>-3.599-2.192</li> <li>0.975</li> <li>0.06KA</li> <li>-0.100</li> <li>0.950</li> <li>-3.599-2.192</li> <li>0.975</li> <li>0.06KA</li> <li>Betulin</li> <li>-1.192</li> <li>0.950</li> <li>-4.087-1.704</li> <li>0.865</li> <li>0.001*</li> <li>PBS</li> <li>-2.645</li> <li>0.996</li> <li>-5.682-0.391</li> <li>0.116</li> <li>OKA</li> <li>-3.37</li> <li>0.950</li> <li>-13.469-7.678</li> <li>0.001*</li> <li>PBS</li> <li>-10.473</li> <li>0.950</li> <li>-13.469-7.578</li> <li>0.001*</li> <li>PBS</li> <li>OKA</li> <li>-11.277</li> <li>0.950</li></ul>  |           |        |                            | Betulin                     | 10.328  | 0.673 | 8.276-12.380    | 0.001*  |
| *Small COX2 PBS Control 2.0367 0.673 0.101-4.205 0.001*<br>OKA+Betulin Control 2.153 0.673 0.101-4.205 0.001*<br>DMSO 3.563 0.706 1.411-5.715 0.001*<br>Betulin 12.365 0.673 10.312-14.417 0.001*<br>PBS 1.722 0.673 -0.310-3.774 0.141<br>OKA 2.037 0.673 -0.015-4.089 0.053<br>Control DMSO 2.745 0.996 -0.291-5.782 0.094<br>Betulin 10.573 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 -2.795-2.995 1.000<br>OKA -0.703 0.950 -3.599-2.192 0.975<br>OKA + Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS 0.2645 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS 0.2645 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.485-0.485 0.019<br>OKA + Betulin -3.937 0.996 -6.974-0.900 0.006*<br>PBS -10.473 0.950 -13.469-7.678 0.001*<br>PBS -10.473 0.950 -13.469-7.678 0.001*<br>PBS -10.473 0.950 -13.469-7.678 0.001*<br>DMSO 2.645 0.996 -0.386-7.578 0.001*<br>DMSO 2.645 0.996 -0.381-9.758 0.001*<br>OKA -11.277 0.950 -14.172-8.381 0.001*<br>OKA -11.277 0.950 -14.660-8.869 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA +Betulin 11.765 0.950 -4.187-1.604 0.750<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-4.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995   |           |        |                            | PBS                         | -0.315  | 0.673 | -2.367-1.737    | 0.997   |
| *Small<br>Htestine<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX4<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>PBS<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>COX2<br>CO |           |        | OVALD (1                   | OKA+Betulin                 | -2.0367 | 0.673 | -4.089-0.015    | 0.053   |
| *Small<br>Intestine<br>COX2 PBS COX2 PBS COX2 PBS COX2 PBS COX2 PBS COX2 PBS COX2 COX4 PBS COX2 PBS COX2 PBS COX2 PBS COX2 COX2 COX4 PBC COX2 PBS COX2 COX2 COX4 PBC COX2 COX2 COX2 COX4 PBC COX2 COX2 COX2 COX4 PBC COX2 PBS COX2 PBS COX2 COX2 COX2 COX4 PBC COX2 COX2 COX2 COX2 COX4 PBC COX2 PBS COX2 PBS COX2 PBS COX2 PBS COX2 COX2 COX2 COX2 COX2 COX2 COX2 COX2  |           |        | OKA+Betulin                | Control                     | 2.153   | 0.6/3 | 0.101-4.205     |         |
| *Small Intestine COX2 PBS Control 12.503 0.075 10.512-14.41/ 0.001*<br>PBS 1.722 0.673 -0.015-4.089 0.053<br>OKA 2.037 0.673 -0.015-4.089 0.053<br>OKA 2.037 0.673 -0.015-4.089 0.053<br>OKA 2.037 0.673 -0.015-4.089 0.001*<br>PBS 0.100 0.950 -2.795-2.995 1.000<br>OKA -0.703 0.950 -3.599-2.192 0.975<br>OKA+Betulin -1.192 0.950 -4.087-1.704 0.806<br>OKA -0.703 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS -2.645 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.485-0.485 0.019<br>OKA +Betulin -3.937 0.996 -6.974-0.900 0.006*<br>PBS -10.473 0.950 -13.369-7.578 0.001*<br>DMSO 7.828 0.996 -10.865-4.859 0.001*<br>DMSO -7.828 0.996 -10.865-4.791 0.001*<br>OKA -11.277 0.950 -14.172-8.381 0.001*<br>OKA -11.277 0.950 -14.172-8.381 0.001*<br>OKA -11.277 0.950 -14.172-8.381 0.001*<br>OKA -11.277 0.950 -14.600-8.869 0.001*<br>OKA -11.277 0.950 -14.172-8.381 0.001*<br>OKA -0.803 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -Betulin -1.272 0.950 -4.187-1.604 0.750<br>OKA -0.803 0.950 -2.192-3.599 0.956<br>OKA+Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA +Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA +Betulin 11.277 0.950 -3.369-2.092 0.956<br>OKA+Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA -0.803 0.950 -2.192-3.599 0.956<br>OKA+Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA -1801 0.703 0.950 -2.192-3.599 0.956<br>OKA+Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA -1801 0.703 0.950 -2.192-3.599 0.956<br>OKA+Betulin 11.277 0.950 -3.384-2.407 0.995<br>OKA+Betulin 11.277 0.950 0.704-4.087 0.806<br>OKA+Betulin 11.270 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 0.900-6.974 0.006*  |           |        |                            | DMSU<br>Datulin             | 3.303   | 0.706 | 1.411-5./15     | 0.001*  |
| *Small<br>HSS 1.122 0.953 -0.305 -0.305 -0.305 -0.408<br>Control DMSO 2.745 0.996 -0.291-5.782 0.094<br>Betulin 10.573 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 -2.795-2.995 1.000<br>OKA -0.703 0.950 -3.599-2.192 0.975<br>OKA + Betulin -1.192 0.950 -4.087-1.704 0.806<br>DMSO Control -2.745 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS -2.645 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.485-0.485 0.019<br>OKA + Betulin -11.573 0.950 -13.469-7.678 0.001*<br>DMSO 7.828 0.996 -10.865-4.791 0.001*<br>DMSO -7.828 0.996 -10.865-4.791 0.001*<br>OKA - 11.277 0.950 -14.172-8.381 0.001*<br>OKA - 0.803 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA + Betulin -1.292 0.950 -4.187-1.604 0.750<br>OKA -0.803 0.950 -3.384-2.407 0.955<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>DMSO 3.347 0.950 -3.384-2.407 0.955<br>DMSO 3.347 0.950 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>DMSO 3.347 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 0.900-6.974 0.006*  |           |        |                            | Detuini                     | 12.303  | 0.075 | 0 330 3 774     | 0.141   |
| *Small COX2 PBS Control DMSO 2.745 0.996 -0.291-5.782 0.094<br>Betulin 10.573 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 -2.795-2.995 1.000<br>OKA -0.703 0.950 -3.599-2.192 0.975<br>OKA + Betulin -1.192 0.950 -4.087-1.704 0.806<br>DMSO Control -2.745 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 -5.782-0.291 0.094<br>Betulin -3.937 0.996 -5.782-0.291 0.094<br>Betulin -3.937 0.996 -6.9740.900 0.006*<br>PBS -10.473 0.950 -13.4697.678 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>PBS -10.473 0.950 -13.3697.578 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>DMSO -7.578 0.950 -14.1728.381 0.001*<br>OKA +Betulin -11.765 0.950 -14.1608.869 0.001*<br>OKA - 0.803 0.950 -3.699-2.092 0.956<br>OKA +Betulin -1.292 0.950 -4.187-1.604 0.750<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 -7.578-1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 -7.578-1.3.369<br>OKA - 0.803 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin -11.92 0.950 -1.704-4.087 0.955<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin -11.927 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin -11.927 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin -0.488 0.950 -3.384-2.407 0.995<br>DMSO 3.349 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 8.869-14.660 0.001*   |           |        |                            | OKA                         | 2 037   | 0.073 | -0.015-4.089    | 0.141   |
| *Small<br>Intestine COX2<br>PBS 0.100 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 7.678-13.469 0.001*<br>PBS 0.100 0.950 7.2795-2.995 1.000<br>OKA - 0.703 0.950 -3.599-2.192 0.975<br>OKA+ Betulin -1.192 0.950 -4.087-1.704 0.806<br>Control -2.745 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS -2.645 0.996 -5.682-0.391 0.116<br>OKA - Betulin -3.937 0.996 -6.4850.485 0.019<br>OKA+ Betulin -10.573 0.950 -13.4697.678 0.001*<br>DMSO -7.828 0.996 -10.865-4.791 0.006*<br>PBS -10.473 0.950 -13.697.578 0.001*<br>OKA - Btulin -11.765 0.950 -14.6608.869 0.001*<br>OKA + Betulin -11.765 0.950 -14.6608.869 0.001*<br>OKA - Betulin 10.473 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA - 0.803 0.950 -3.699-2.092 0.956<br>OKA + Betulin -1.292 0.950 -4.187-1.604 0.750<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>OKA - Control 0.703 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.950<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.951  |           |        | Control                    | DMSO                        | 2.037   | 0.075 | -0.291-5.782    | 0.094   |
| *Small<br>Intestine         COX2<br>COX2         PBS         0.100         0.950         -2.795-2.995         1.000           OKA         -0.703         0.950         -3.599-2.192         0.975           OKA+Betulin         -1.192         0.950         -3.599-2.192         0.975           OKA+Betulin         -1.192         0.950         -4.0871.704         0.806           DMSO         Control         -2.745         0.996         4.791-10.865         0.001*           PBS         -2.645         0.996         -5.682-0.391         0.116           OKA         -3.449         0.996         -6.485-0.485         0.019           OKA+Betulin         -3.937         0.996         -6.6974-0.900         0.006*           DMSO         -7.828         0.996         -10.865-4.791         0.001*           DMSO         -7.828         0.996         -10.865-4.791         0.001*           DMSO         -7.828         0.996         -10.865-4.791         0.001*           OKA         -11.277         0.950         -14.660-8.869         0.001*           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         -2.192-  |           |        | Control                    | Betulin                     | 10.573  | 0.950 | 7.678-13.469    | 0.001*  |
| *Small Intestine COX2 PBS OKA -0.703 0.950 -3.599-2.192 0.975<br>OKA+ Betulin -1.192 0.950 -4.087-1.704 0.806<br>Control -2.745 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS -2.645 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.4850.485 0.019<br>OKA+ Betulin -3.937 0.996 -6.9740.900 0.006*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>PBS -10.473 0.950 -13.4697.678 0.001*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>PBS -10.473 0.950 -13.3697.578 0.001*<br>OKA + Betulin -11.765 0.950 -14.6608.869 0.001*<br>OKA + Betulin -11.765 0.950 -14.6608.869 0.001*<br>OKA + Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA + Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA +Betulin 11.277 0.950 4.187-1.604 0.750<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA +Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA+Betulin 11.277 0.950 -4.187-1.604 0.750<br>OKA -0.803 0.950 -3.3699-2.092 0.956<br>OKA+Betulin 11.277 0.950 -3.381-4.172 0.001*<br>PBS 0.803 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995<br>OKA+Betulin 11.276 0.950 -1.704-4.087 0.806<br>DMSO 3.937 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 8.869-14.660 0.001*   |           |        |                            | PBS                         | 0.100   | 0.950 | -2.795-2.995    | 1.000   |
| *Small Intestine COX2 PBS COX4 + Betulin -1.192 0.950 -4.087-1.704 0.806<br>Control -2.745 0.996 -5.782-0.291 0.094<br>Betulin 7.820 0.996 4.791-10.865 0.001*<br>PBS -2.645 0.996 -5.682-0.391 0.116<br>OKA -3.449 0.996 -6.4780.485 0.019<br>OKA+ Betulin -3.937 0.996 -6.9740.900 0.006*<br>DMSO -7.828 0.996 -10.8654.791 0.001*<br>PBS -10.473 0.950 -13.4697.678 0.001*<br>PBS -10.473 0.950 -13.6697.578 0.001*<br>OKA -11.765 0.950 -14.1728.381 0.001*<br>OKA -8tetulin -11.765 0.950 -14.1608.869 0.001*<br>OKA + Betulin 10.473 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA - 0.803 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995   |           |        |                            | OKA                         | -0.703  | 0.950 | -3.599-2.192    | 0.975   |
| *Small<br>Intestine         COX2         Control<br>Betulin         -2.745<br>7.820         0.996<br>0.996         -5.782-0.291<br>4.791-10.865         0.001*<br>0.001*           PBS         -2.645         0.996         -5.682-0.391         0.116           OKA         -3.449         0.996         -6.682-0.391         0.116           OKA         -3.449         0.996         -6.4850.485         0.019           OKA+Betulin         -3.937         0.996         -6.9740.900         0.006*           Control         -10.573         0.950         -13.4697.678         0.001*           DMSO         -7.828         0.996         -10.8654.791         0.001*           DMSO         -7.828         0.996         -10.8654.791         0.001*           OKA         -11.277         0.950         -13.3697.578         0.001*           OKA         -11.277         0.950         -14.1728.381         0.001*           OKA+Betulin         10.473         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         -3.699-2.092         0.956           OKA         -8030         0.950  |           |        |                            | OKA+ Betulin                | -1.192  | 0.950 | -4.087-1.704    | 0.806   |
| *Small<br>Intestine         COX2<br>COX2         PBS         -2.645         0.996         -4.791-10.865         0.001*           *Small<br>Intestine         Betulin         7.820         0.996         -5.682-0.391         0.116           OKA         -3.449         0.996         -6.4850.485         0.019           OKA+ Betulin         -3.937         0.996         -6.9740.900         0.006*           OKA+ Betulin         -10.573         0.950         -13.4697.678         0.001*           DMSO         -7.828         0.996         -10.8654.791         0.001*           DMSO         -7.828         0.996         -10.8654.791         0.001*           OKA         -11.277         0.950         -14.1728.381         0.001*           OKA         -11.277         0.950         -14.6608.869         0.001*           OKA         -0.100         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         -3.699-2.092         0.956           OKA         -0.803         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996  |           |        | DMSO                       | Control                     | -2.745  | 0.996 | -5.782-0.291    | 0.094   |
| $\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$   |           |        |                            | Betulin                     | 7.820   | 0.996 | 4.791-10.865    | 0.001*  |
| *Small<br>Intestine         COX2<br>COX2         PBS         OKA<br>Control         -3.449<br>-10.573         0.996<br>0.996         -6.4850.485<br>-6.9740.900         0.006*<br>0.006*           *Small<br>Intestine         COX2         Betulin         Control         -10.573         0.950         -13.4697.678         0.001*           *Small         DMSO         -7.828         0.996         -10.8654.791         0.001*           VBS         -10.473         0.950         -13.3697.578         0.001*           OKA         -11.277         0.950         -14.16608.869         0.001*           OKA         -11.277         0.950         -14.6608.869         0.001*           OKA         -11.277         0.950         -14.6608.869         0.001*           OKA         -0.100         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         -3.699-2.092         0.956           OKA         -0.803         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         -3.38  |           |        |                            | PBS                         | -2.645  | 0.996 | -5.682-0.391    | 0.116   |
| *Small<br>Intestine         COX2<br>COX2         PBS         Control<br>Control         -10.573<br>-10.473         0.996<br>0.996         -6.9740.900<br>-13.4697.678         0.001*<br>0.001*           *Small<br>Intestine         COX2         PBS         -10.473         0.950         -13.4697.678         0.001*           *Small         COX2         PBS         -10.473         0.950         -13.3697.578         0.001*           %Small         COX2         PBS         -0.10473         0.950         -14.1728.381         0.001*           OKA+ Betulin         -11.277         0.950         -14.6608.869         0.001*           OKA+ Betulin         -0.100         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         7.578-13.369         0.001*           OKA         -0.803         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.192-3.599         0.975           DMSO  |           |        |                            | OKA                         | -3.449  | 0.996 | -6.4850.485     | 0.019   |
| Betulin         Control         -10.573         0.950         -13.4697.678         0.001*           DMSO         -7.828         0.996         -10.8654.791         0.001*           PBS         -10.473         0.950         -13.3697.578         0.001*           OKA         -11.277         0.950         -14.1728.381         0.001*           OKA         -11.277         0.950         -14.6608.869         0.001*           OKA+Betulin         -11.765         0.950         -14.6608.869         0.001*           OKA+Betulin         -0.100         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         7.578-13.369         0.001*           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -3.384-2.407   |           |        |                            | OKA+ Betulin                | -3.937  | 0.996 | -6.9740.900     | 0.006*  |
| *Small<br>Intestine COX2<br>Intestine COX2<br>PBS -10.473 0.950 -13.3697.578 0.001*<br>OKA -11.277 0.950 -14.1728.381 0.001*<br>OKA + Betulin -11.765 0.950 -14.6608.869 0.001*<br>Control -0.100 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA + Betulin -1.292 0.950 -4.187-1.604 0.750<br>OKA Control 0.703 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin -1.292 0.950 -1.704-4.087 0.806<br>DMSO 3.937 0.950 -1.704-4.087 0.806<br>DMSO 3.937 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 8.869-14.660 0.001*   |           |        | Betulin                    | Control                     | -10.573 | 0.950 | -13.4697.678    | 0.001*  |
| *Small<br>Intestine       COX2       PBS       -10.4/3       0.950       -13.3697.578       0.001*         *Small<br>Intestine       COX2       PBS       OKA       -11.277       0.950       -14.1728.381       0.001*         OKA+Betulin       -11.765       0.950       -14.6608.869       0.001*         OKA       Control       -0.100       0.950       -2.995-2.795       1.000         DMSO       2.645       0.996       -0.391-5.682       0.116         Betulin       10.473       0.950       7.578-13.369       0.001*         OKA       -0.803       0.950       -3.699-2.092       0.956         OKA       Control       0.703       0.950       -2.192-3.599       0.975         DMSO       3.449       0.996       0.7412-6.485       0.019*         Betulin       11.277       0.950       8.381-14.172       0.001*         OKA+Betulin       -0.488       0.950       -3.384-2.407       0.995         OKA+Betulin       -0.488       0.950       -3.384-2.407       0.995         OKA+Betulin       -0.488       0.950       -3.384-2.407       0.995         OKA+Betulin       1.192       0.950       -1.704-4.087       0.806   |           |        |                            | DMSO                        | -/.828  | 0.996 | -10.8654.791    |         |
| *Small<br>Intestine COX2 PBS OKA -11.277 0.950 -14.1728.381 0.001*<br>OKA + Betulin -11.765 0.950 -14.6608.869 0.001*<br>Control -0.100 0.950 -2.995-2.795 1.000<br>DMSO 2.645 0.996 -0.391-5.682 0.116<br>Betulin 10.473 0.950 7.578-13.369 0.001*<br>OKA -0.803 0.950 -3.699-2.092 0.956<br>OKA Control 0.703 0.950 -2.192-3.599 0.975<br>DMSO 3.449 0.996 0.7412-6.485 0.019*<br>Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -2.092-3.699 0.956<br>OKA+Betulin 11.277 0.950 8.381-14.172 0.001*<br>PBS 0.803 0.950 -3.384-2.407 0.995<br>OKA+Betulin -0.488 0.950 -3.384-2.407 0.995<br>OKA+Betulin 11.122 0.950 -1.704-4.087 0.806<br>DMSO 3.937 0.950 0.900-6.974 0.006*<br>Betulin 11.765 0.950 8.869-14.660 0.001*  |           |        |                            | PBS                         | -10.4/3 | 0.950 | -13.3097.378    | 0.001*  |
| *Small<br>Intestine         COX2         PBS         Control         -0.100         0.950         -14.0000.309         0.001           Intestine         PBS         Control         -0.100         0.950         -2.995-2.795         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         7.578-13.369         0.001*           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           DMSO         3.449         0.996         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660         0.006*  | *0 11     |        |                            | OKA<br>OK A+ Betulin        | -11.277 | 0.950 | -14.1/20.301    | 0.001*  |
| Intestine         I bb         Control         0.100         0.130         12.175         1.000           DMSO         2.645         0.996         -0.391-5.682         0.116           Betulin         10.473         0.950         7.578-13.369         0.001*           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         -0.803         0.950         -4.187-1.604         0.750           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         11.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660         0.006*  | *Small    | COX2   | PRS                        | Control                     | -0.100  | 0.950 | -14.0008.809    | 1 000   |
| DHIO         D.013         0.013         0.013         0.014           Betulin         10.473         0.950         7.578-13.369         0.001*           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660         0.006*   | Intestine |        | 105                        | DMSO                        | 2 645   | 0.996 | -0 391-5 682    | 0.116   |
| OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         -0.803         0.950         -3.699-2.092         0.956           OKA         Control         -1.292         0.950         -4.187-1.604         0.750           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         -0.488         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660         0.006*   |           |        |                            | Betulin                     | 10 473  | 0.950 | 7 578-13 369    | 0.001*  |
| OKA         Betulin         -1.292         0.950         -4.187-1.604         0.750           OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485 <b>0.019*</b> Betulin         11.277         0.950         -2.092-3.699         0.956           OKA+Betulin         0.603         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         0.011         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660 <b>0.006*</b>   |           |        |                            | OKA                         | -0.803  | 0.950 | -3.699-2.092    | 0.956   |
| OKA         Control         0.703         0.950         -2.192-3.599         0.975           DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         0.011         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         8.869-14.660         0.001*  |           |        |                            | OKA+ Betulin                | -1.292  | 0.950 | -4.187-1.604    | 0.750   |
| DMSO         3.449         0.996         0.7412-6.485         0.019*           Betulin         11.277         0.950         8.381-14.172         0.001*           PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         0.900-6.974         0.006*           Betulin         11.765         0.950         8.869-14.660         0.001*   |           |        | OKA                        | Control                     | 0.703   | 0.950 | -2.192-3.599    | 0.975   |
| Betulin11.2770.9508.381-14.1720.001*PBS0.8030.950-2.092-3.6990.956OKA+Betulin-0.4880.950-3.384-2.4070.995OKOTrol1.1920.950-1.704-4.0870.806DMSO3.9370.9500.900-6.9740.006*Betulin11.7650.9508.869-14.6600.001*   |           |        |                            | DMSO                        | 3.449   | 0.996 | 0.7412-6.485    | 0.019*  |
| PBS         0.803         0.950         -2.092-3.699         0.956           OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         0.900-6.974         0.006*           Betulin         11.765         0.950         8.869-14.660         0.001*   |           |        |                            | Betulin                     | 11.277  | 0.950 | 8.381-14.172    | 0.001*  |
| OKA+Betulin         -0.488         0.950         -3.384-2.407         0.995           OKA+Betulin         Control         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         0.900-6.974         0.006*           Betulin         11.765         0.950         8.869-14.660         0.001*  |           |        |                            | PBS                         | 0.803   | 0.950 | -2.092-3.699    | 0.956   |
| OKA+Betulin         Control         1.192         0.950         -1.704-4.087         0.806           DMSO         3.937         0.950         0.900-6.974 <b>0.006*</b> Betulin         11.765         0.950         8.869-14.660 <b>0.001*</b>  |           |        | OKA+Betulin                | OKA+Betulin                 | -0.488  | 0.950 | -3.384-2.407    | 0.995   |
| DMSO         3.937         0.950         0.900-6.974         0.006*           Betulin         11.765         0.950         8.869-14.660         0.001*   |           |        |                            | Control                     | 1.192   | 0.950 | -1.704-4.087    | 0.806   |
| Betulin 11.765 0.950 8.869-14.660 <b>0.001</b> *   |           |        |                            | DMSO                        | 3.937   | 0.950 | 0.900-6.974     | 0.006*  |
|  |           |        |                            | Betulin                     | 11.765  | 0.950 | 8.869-14.660    | 0.001*  |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$   |           |        |                            | rbs<br>OK A+Batulin         | 1.292   | 0.950 | -1.004-4.18/    | 0.750   |

\*: P<0.05; DMSO: Dimetil sülfoksit; OKA: Okadaik asit; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; PBS: Phosphate Buffer Saline.

The distribution of hippocampal COX proteins was tested in the AD rat model. The results showed that Betulin had different histochemical scores in different tissues (H-score =  $(0 \times P_0) + (1 \times P_1) + (2 \times P_2) + (3 \times P_3)$ ). The results showed that the most significant effect of COX1 was in the heart, moderately in the kidney, and to a lesser extent in the small intestine. COX2 showed the most significant effect in the kidney, a moderate impact on the heart, and finally, a low effect in the small intestine tissue. H-scores for AD and treatment group were as follows. In the structural histological evaluation of kidney tissue, a high expression of COX1 was observed in tubular,

mesangial cells and podocytes (Figure 2A), and a relatively lower rate of uptake for COX2 was observed (Figure 2G). While protein retention was not observed for COX1 after Betulin treatment (Figure 2B), it was found to decrease for COX2 (Figure 2H). Heart tissue analyses showed focal myonecrosis and degeneration in the AD model for COX1 (Figure 2C) and COX2 (Figure 2I). Low protein levels were detected after Betulin treatment (Figure 2D, 2J). Mild and moderate defects observed in AD heart tissue could not be seen after Betulin treatment. In the small intestine tissue, expression of COX1 and COX2 in the ileum was detected in the AD group (Figure 2E, 2K). A decrease in protein expression



**Figure 2.** Immunohistochemical staining of COX1 and COX2 in rat kidney, heart, and small intestine  $(100\times)$ . A: Kidney- Okadaic acid showing high expression of COX1 in tubular cell, mesangial cell, and podocytes (black arrow); B: Kidney- Okadaic acid + Betulin group this group showing no expression for COX1; C: Heart- Okadaic acid showing high expression in heart muscle cells for COX1 (black arrow); D: Heart- Okadaic acid + Betulin group after Betulin treatment, COX1 expression was observed in low amounts in heart muscle cells; E: Small intestine-Okadaic acid showing high expression for COX1; in ileum section; F: Small intestine-Okadaic acid + Betulin group, Solving low expression for COX2; in kidney-Okadaic acid showing high expression of COX2; H: Kidney-Okadaic acid + Betulin group, showing low expression for COX2 in kidney section; I: Heart-Okadaic acid showing high expression in heart muscle cells; K: Small intestine-Okadaic acid showing high expression for COX2 in kidney-Okadaic acid showing high expression for COX2; H: Kidney-Okadaic acid showing high expression for COX2; J: Heart- Okadaic acid + Betulin group, showing low expression for COX2 expression was observed in low amounts in heart muscle cells; K: Small intestine-Okadaic acid showing high expression for COX2 in kidney-Okadaic acid showing in heart muscle cells; K: Small intestine-Okadaic acid showing high expression for COX2 expression was observed in low amounts in heart muscle cells; K: Small intestine-Okadaic acid showing high expression for COX2 in ileum section; L: Small intestine-Okadaic acid + Betulin group, COX2 expression was observed in low amounts in the ileum; MF: muscle fiber; \*: mild defect; \*\*: moderate defect.

was observed after Betulin treatment (Figure 2F, 2L).

#### DISCUSSION AND CONCLUSION

The relationship between dementia and acute organ dysfunction threatens the patient's life and affects mortality. In chemically OKA-induced AD, excessive COX1 and COX2 gene expressions in kidney, heart, and small intestine tissues indicate their structural and functional integrity is in danger. The literature mostly mentions the predominant effect of COX2 dysfunction in the kidney. However, there is no definitive comment for COX1. When the function of COX-1 in the kidney is examined, it is seen that it plays a role in hemodynamic regulation.<sup>12</sup> Therefore, afferent and efferent COX1 dysfunction may disrupt renal hemodynamics and unbalance the glomerular filtration rate. The improvement of COX1 and COX-2 levels under the influence of Betulin indicates that this metabolism can be regulated by targeting COXs. The possibility of damage via COXs is supported by a mouse study. The fact that gene expressions can be decreased by Betulin indicates that the COX pathway may be Betulin's target. Ensuring homeostasis related to the absorption of ions in renal functions is regulated by many physiological mechanisms. Studies have shown that protein phosphatase 2 (PP2A) is responsible for maintaining ion channels and homeostasis.<sup>13</sup> In addition to its function in Na-Cl-dependent transporter systems, PP2A accelerates the flow of Na-K-ATPase from the intracellular system to the basal-lateral membrane in human adenocarcinoma cells. In this mechanism, okadaic acid inhibits the increase in Na-K-ATPase activity as a selective inhibitor of PP2A.<sup>14</sup> Two features observed in the initial pathology of chronic kidney disease, glomerulosclerosis, and tubulointerstitial fibrosis, are associated with microvascular endothelial cell dysfunction.<sup>15</sup> Okadaic acid reduces the effect of PP2A on the endothelial cell remodelling process. Thus, okadaic acid harms kidney homeostasis, both structurally and functionally. In our study, it is possible to regulate okadaic acid-induced kidney damage during the AD process by managing COX enzymes with the effect of Betulin. A study supports the accuracy of this idea. The study showed that the left ventricle in AD patients was thicker than in patients without the disease. The reason for this thickness is that Ab plaques in the AD brain accumulate in the same form in the ventricle. Ventricular thickening impedes blood flow, which can result in cardiovascular problems and a higher risk of heart attack and stroke.<sup>16</sup> PP2A function is also present in the heart. It is a phosphatase that modulates Ca<sup>2+</sup> utilization as a channel regulator. In our study, heart tissue analyses show that cellular COX protein accumulations in the okadaic acid AD model are partially

reduced by the effect of Betulin and that damage can be reduced by regulating COX metabolism with Betulin treatment. Therefore, it indicates that partial recovery of heart functions is possible. The most well-known effect of okadaic acid on the intestinal system is that it stimulates the phosphorylation of proteins that control Na release in cells, increases protein phosphorylation due to solute permeability, and therefore causes fluid loss. The data we obtained in our study may indicate that structural differentiation and permeability in intestinal cells may change as a result of overexpression of COXs, causing them to become partially or completely dysfunctional. Reducing the amount of protein with the effect of Betulin can restore this function.

Peripheral inflammation outside the CNS in AD is a risk factor for the disease.<sup>17</sup> The contribution of these processes to the neurology of AD is unclear. However, acute inflammation creates a temporal immune challenge that will cause tissue damage.<sup>18</sup> COXs may have a role in AD's blood-brain barrier and neuro-immune connection.<sup>19,20</sup> The impact of peripheral inflammation on brain function in the neuro-immune connection is associated with increased inflammatory proteins in the blood.<sup>21</sup>These proteins activate endothelial cells, resulting in vascular inflammation.<sup>22</sup> Proinflammatory transcription factors that are subsequently activated cause the expression of the same molecules in the brain.<sup>23</sup> At the same time, peripheral inflammatory proteins can be transported to the brain by age-dependent caveolar transcytosis.<sup>24</sup> The vagus nerve also plays a role in this event.<sup>25</sup> It transmits inflammation signals from the intestine, liver, lung, and other organs to the brain. These signals can activate inflammatory proteins and receptors in glial cells via the solitary nucleus.<sup>17</sup> A sudy shows that sepsis following infection threatens acute organ failure, aggravates AB accumulation and triggers systemic inflammation that causes AD to progress.<sup>26</sup> Damage to the vascular system is associated with an increased risk of AD. Neuroinflammation and systemic overstimulation are among the etiologies of AD and lead to neuronal death due to synaptic dysfunction. There is increasing evidence that infection and organ disorders cause peripheral AB and neurovascular dysfunction.<sup>27</sup>

In conclusion, Betulin may be a COX inhibitor candidate like other potent NSAIDs. Therefore, it may have a role in AD and treating specific damage to each other's organs. Most importantly, it can be mentioned that it has a potential effect on all mechanisms under the COX effect. The idea that organ damage causes AD may change direction towards the idea that AD can also cause organ damage. The limitation of this study is the analysis of structural changes and cellular damage in 3 tissues. Additional studies may help elucidate the extent of textural damage and its association with genes and histology. In addition, all organ functions are affected by AD, which involves inflammatory processes, and not only is the brain the target of treatment. The COXs expressed in this study have significant potential as therapeutic targets. Although many alternatives exist, gene-level editing tools can be guiding in this regard.

*Ethics Committee Approval:* The work described in this article has been carried out by the Gaziantep University Experimental Animals Local Ethics Committee (Date:21.09.2023, decision no: 2023-29). *Conflict of Interest:* No conflict of interest was declared by the authors.

*Author Contributions:* Concept – ASB; Supervision – ASB, ŞGY; Materials – ASB, ŞGY; Data Collection and/or Processing – ASB, ŞGY; Analysis and/or Interpretation – ASB, ŞGY; Writing – ASB, ŞGY. *Peer-review:* Externally peer-reviewed.

*Financial Support:* This research was supported by the Gaziantep University Scientific Research Projects Unit with the project code TF. HZP. 23.43.

#### REFERENCES

- Ma C, Long H. Protective effect of betulin on cognitive decline in streptozotocin (STZ)induced diabetic rats. Neurotoxicology. 2016;57:104-111. doi:10.1016/ j.neuro.2016.09.009
- Kamat PK, Rai S, Nath C. Okadaic acid induced neurotoxicity: an emerging tool to study Alzheimer's disease pathology. Neurotoxicology. 2013;37:163-172. doi:10.1016/ j.neuro.2013.05.002
- Kamat PK, Tota S, Saxena G, et al. Okadaic acid (ICV) induced memory impairment in rats: a suitable experimental model to test anti-dementia activity. Brain Res. 2010;1309:66-74. doi:10.1016/j.brainres.2009.10.064
- Adepoju FO, Duru KC, Li E, Kovaleya EG, Tsurkan MV. Pharmacological potential of betulin as a multitarget compound. Biomolecules. 2023;13(7):1105. doi:10.3390/biom13071105
- Amiri S, Dastghaib S, Ahmadi M, et al. Betulin and its derivatives as novel compounds with different pharmacological effects. Biotechnol Adv. 2020;38:107409. doi:10.1016/ j.biotechadv.2019.06.008
- Takibayeva AT, Zhumabayeva GK, Bakibaev AA, et al. Methods of analysis and identification of betulin and its derivatives. Molecules. 2023;28 (16):5946. doi:10.3390/molecules28165946
- Faki Y, Er A. Different chemical structures and physiological/pathological roles of cyclooxygenases. Rambam Maimonides Med J. 2021;12 (1):1-13. doi:10.5041/RMMJ.10426

- Adelizzi RA. COX-1 and COX-2 in health and disease. J Am Osteopath Assoc. 1999;99(11):7-12. doi:10.7556/jaoa.1999.03
- Takeuchi K, Tanaka A, Kato S, Amagase K, Satoh H. Roles of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. Clin Chim Acta. 2010;411(7-8):459-466. doi:10.1016/j.eca.2009.12.026
- Rio DC, Ares M, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb Protoc. 2010;2010(6):534. doi:10.1101/pdb.prot5439
- 11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the  $2-\Delta\Delta CT$  method. Methods. 2001;25(4):402-408. doi:10.1006/meth.2001.1262
- 12. Moro MG, Sánchez PKV, Lupepsa AC, Baller EM, Franco GCN. Cyclooxygenase biology in renal function-literature review. Revista Colombiana de Nefrología. 2017;4(1):27-37. doi:10.22265/acnef.4.1.263
- 13. Gu M, Tan M, Zhou L, et al. Protein phosphatase 2Acα modulates fatty acid oxidation and glycolysis to determine tubular cell fate and kidney injury. Kidney Int. 2022;102(2):321-336. doi:10.1016/j.kint.2022.03.024
- 14. Shao L, Ma Y, Fang Q, et al. Role of protein phosphatase 2A in kidney disease. Exp Ther Med. 2021;22(5):1-10. doi:10.3892/ etm.2021.10671
- 15. Nogueira A, Pires MJ, Oliveira PA. Pathophysiological mechanisms of renal fibrosis: a review of animal models and therapeutic strategies. In vivo. 2017;31(1):1-22. doi:10.21873/invivo.11019
- 16. Troncone L, Luciani M, Coggins M, et al. Aβ amyloid pathology affects the hearts of patients with Alzheimer's disease: mind the heart. Am J Cardiol. 2016;68(22):2395-2407. doi:10.1016/ j.jacc.2016.08.073
- 17. Walker KA, Le Page LM, Terrando N, et al. The role of peripheral inflammatory insults in Alzheimer's disease: a review and research roadmap. Mol Neurodegener. 2023;18(1):1-19. doi:10.1186/s13024-023-00627-2
- Megha KB, Joseph X, Akhil V, Mohanan PV. Cascade of immune mechanism and consequences of inflammatory disorders. Phytomedicine. 2021;91:153712. doi:10.1016/j.phymed.2021.153712
- 19. Yang C, Yang Y, DeMars KM, Rosenberg GA, Candelario JE. Genetic deletion or pharmacological inhibition of cyclooxygenase-2 reduces blood -brain barrier damage in experimental ischemic stroke. Front Neurol. 2020;11:887. doi:10.3389/ fneur.2020.00887
- 20. Choi SH, Aid S, Bosetti F. The distinct roles of

cyclooxygenase-1 and-2 in neuroinflammation: implications for translational research. Trends Pharmacol Sci. 2009; 30(4):174-181. doi:10.1016/j.tips.2009.01.002

- 21. Sun Y, Koyama Y, Shimada S. Inflammation from peripheral organs to the brain: how does systemic inflammation cause neuroinflammation. Front Aging Neurosci. 2022;14:903455. doi:10.3389/fnagi.2022.903455
- 22. Immanuel J, Yun S. Vascular Inflammatory Diseases and Endothelial Phenotypes. Cells. 2023;12(12):1640. doi:10.3390/cells12121640
- Tiwari PC, Pal R. The potential role of neuroinflammation and transcription factors in Parkinson disease. Dialogues Clin Neurosci. 2017;19(1):71-80. doi:10.31887/DCNS.2017.19.1/rpal
- 24. Villaseñor R, Lampe J, Schwaninger M, Collin L. Intracellular transport and regulation of transcytosis across the blood-brain barrier. Cell Mol Life Sci. 2019;76:1081-1092. doi:10.1007/ s00018-018-2982-x
- 25. Wang L, Gao F, Wang Z, et al. Transcutaneous auricular vagus nerve stimulation in the treatment of disorders of consciousness: mechanisms and applications. Front Neuroenergetics. 2023;17:1286267. doi:10.3389/ fnins.2023.1286267
- 26. Gasparotto J, Girardi CS, Somensi N, et al. Receptor for advanced glycation end products mediates sepsis-triggered amyloid-β accumulation, Tau phosphorylation, and cognitive impairment. J Biol Chem. 2018;293(1):226-244. doi:10.1074/jbc.M117.786756
- 27. Nelson, Amy R. Peripheral pathways to neurovascular unit dysfunction, cognitive impairment, and Alzheimer's Disease. Frontiers in aging neuroscience. 2022;14:858429. doi:10.3389/fnagi.2022.858429