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The Effect Ofgenistein in Various Doses At the Content of Cyclooxygenase 2 (Cox-2) Inzalirperitoneal of Endometriosis Mice Model

Pengaruh Genistein Berbagai Dosis
Terhadap Kadar Siklooksigenase 2 (Cox-2) Pada Zalir Peritonealmencit Modelendometriosis

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Abstract:
Objective: *To Prove the effect of genistein in variousdosesat the content of cyclooxygenase 2 (COX-2) in peritoneal tract of endometriosis mice model.*
Method: *This study used atrue experimentaldesignin vivo atfemale mice(Musmusculus) withexperimentaldesignPost-Test Only With ControlGroup Design. Involveseightgroups :negative control group (healthy micewithoutgivengenistein), positive control group(mice model ofendometriosiswithoutgivengenistein) and thetreatment groupisthe group thatwas givena variety ofdifferent dosesof genistein: 50mg/day, 100mg/day, 200mg/day, 300mg/day, 400mg/dayand500mg/day. This research was conductedat the Laboratory ofPhysiologyof the Faculty ofMedicine, Universityof BrawijayaandReproductivePhysiology LaboratoryEmbryologiFaculty of Veterinary Medicine, Airlangga University Surabayasampleof a studyusingmice(Musmusculus) endometriosisfemalemodelsas much as32 head, with2-3 monthsof ageand body weight20-30grams. Zalirperitoneumis then takenandput ina tubeto be processedin order tomeasurelevels ofcyclooxygenase 2 (COX-2)Measurements ofCOX-2usingColorimetricCyclooxygenaseKit(Assay Design).*
Result: *that there is a significant difference in the mean levels of cyclooxygenase-2(COX-2) between the positive control group (1.23±0.14^{a}μM) with the administration of genistein treatment group 50 mg (1.01±0.10^{b}μM), with 100 mg of genistein (0.89±0.09^{b}μM), with 200 mg of genistein (0.68±0.14^{c}μM), with 300 mg of genistein (0.58±0.08^{cd}μM), with 400 mg of genistein (0.40±0.11^{d}μM), and also with 500 mg of genistein (0.44±0.11^{d}μM).*
Conclusion: *Givinggenisteincanreduce levels ofcyclooxygenase-2(COX-2)in zalirperitonealmicemodelsendometriosis.*
Keywords: *Cyclooxygenase-2(COX-2), genistein, endometriosis*

Abstrak:
Tujuan : Membuktikan pengaruhpemberian genistein berbagai dosis terhadap kadar Siklooksigenase-2 (COX-2) pada zalir peritoneal mencit model endometriosis.
Metode : Penelitianinimenggunakandesaineksperimenmurni(true eksperimental)secarain vivopadamencit(Musmusculus)betinadenganrancanganpenelitianPost-Test Only With Control Group Design. Melibatkan 8 kelompokyaitukelompokkontrolnegatif (mencitsehattanpadiberikangenistein), kelompokkontrolpositif (model mencit endometriosis tanpadiberikangenistein) dankelompokperlakuanyaitukelompok yang diberikangenisteinberbagaidosis yang berbeda: 50 mg/hari, 100mg/hari, 200mg/hari, 300mg/hari, 400mg/haridan 500 mg/hari.Penelitianinidilaksanakan di LaboratoriumFisiologiFakultasKedokteranUniversitasBrawijaya Malang danLaboratoriumFisiologiReproduksiEmbryologiFakultasKedokteranHewanUniversitasAirlangga Surabaya-Sampelpeneltianmenggunakanmencit(Musmusculus)betina model endometriosis sebanyak 32 ekor, denganusia 2-3 bulandanberatbadan 20-30 gram.Zalir peritoneum kemudiandiambildandimasukkankedalamtabunguntukdiprosesgunapengukurankadarsiklooksigenase-2 .Pengukuran kadar COX-2 menggunakan Colorimetric Cyclooxygenase-2 Kit (Assay Design)
Hasil: bahwaadaperbedaan yang bermaknarerata kadar siklooksigenase-2 (COX-2) antarakelompokkontrolpositif (1.23±0.14^{a}μM)dengankelompokperlakuanpemberiangenistein 50 mg (1.01±0.10^{b}μM), dengan genistein 100 mg (0.89±0.09^{b}μM), dengan genistein 200 mg (0.68±0.14^{c}μM), dengan genistein 300 mg (0.58±0.08^{cd}μM), dengan genistein 400 mg (0.40±0.11^{d}μM), dan juga dengan genistein 500 mg (0.44±0.11^{d}μM).

Kesimpulan:Pemberiangenisteindapatmenurunkankadarsiklooksigenase-2 (COX-2) padazalir peritoneal mencit model endometriosis.
Kata kunci:Cyclooxygenase 2 (COX-2), genistein, endometriosis

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I. Introduction

Endometriosisbecomeone ofthe majorproblemsof re-production todaybecause ofthe incidence ofthis diseaseis quite high.Endometriosisaffects6-10% of women at reproductive agefrom allethnicand social groups. Found1 in 10women of reproductive age(15-49 years), or about 176millionwomenworldwide are infected withendometriosis.[1,2,9]The incidenceof endometriosisamong allpelvicsurgeryrangedabout 5-15%, andthat interestwas found inthe unmarried womenandyoung age. Universallyendometriosiswillcausecomplaintsof dysmenorrhea, dyspareunia, dysuria, chronicabdominalpain, pelvic painandpain ondefecation.[3,4,5]

Progression ofendometriosisimplantsis influencedby the estrogen hormone(estrogen dependent).The presenceandgrowth ofendometriosiscellsbeginsat the time ofretrograde menstruation, endometrial cellsareshedalong withmenstrual bloodandmetaboliteswill reverse direction(reflux) passes throughthe fallopian tubesthen into theperitonealcavitycausesendometrialcells andtissueattached to theperitonealsurface.[7,8]Theendometriosis tissueesecreteestrogenwhichis estrogenicinvolvinglocalP450aromataseresulting in decreased17β-hydroxysteroiddehydrogenase(17β-HSD) type-2. The enzymearomatasetransforma weak estrogen(estrone) intoa strongestrogen(estradiol).As well asthe normalendometrium, implantationofendometriosis tissuealso hasthe steroidreceptorsER-α andER-β tothe estrogen producedin the bodythatbinds toits receptor.[18]

In the development ofperitonealendometriosis, immune cellsappearin theperitoneal cavityas a result ofinflammation. Amongimmune cells, macrophages are thepredominantcell typein theperitoneal cavity,macrophagesare involved inphagocytosismainlycleaningdebrisretrogradeendometrialcells.Supposedlyperitoneal macrophagescapable to remove debrisretrogradeendometrialcells. But inthe case ofendometriosis, macrophagesfailtoperformthe function ofphagocytosisinretrogradeendometrial tissueand thus allowthe implantationand proliferation ofendometriosis lesions.[5,7]

Advances ininvestigating themolecular mechanismsof pathologicalprocessesendometriosis, whichprostaglandinE2(PGE2) plays animportantroleinprosurvivalandimmuneeffects. This isbecause theconcentration ofPGE2was foundinthe peritonealfluidof womenwithendometriosisis muchgreaterthanin theperitonealfluidof womenwith noendometriotsis. Increased production of prostaglandin E2 100-fold higher in endometriosis due to increased activity of the expression of the enzyme cyclooxygenase-2 (COX-2) induced by IL-1β, TNF-α, MIF and proinflammatory agents. The enzyme cyclooxygenase-2 is the first enzyme involved in converting arachidonic acid (AA) into prostaglandins. The growth of endometriosis involves the role of PGE2. PGE2 is a versatile eicosanoids that have many physiological and pathological functions of a disease. PGE2 involved to play an important role in the development of endometriosis. PGE2 plays a role in regulating the pathophysiological processes including immune suppression, antiapoptosis, angiogenesis and cell proliferation during the development of endometriosis.[77]

Estrogen induces the production of pro-inflammatory cytokin (TNF-α, IL-β, TGF-β and COX2), which subsequently activates the transcription factor NF-κβ. Estradiol binds to ER-α and ER-β, forming bonds of estrogen and estrogen-receptor complex then binds to a specific piece of DNA called a promoter ERE genes in the nucleus.[16,18]Toactivatethe transcription process, bondingof estrogenandestrogen-receptorcomplextobind to theEREco-regulatory proteinsthatco-activator proteins.[7,12]Transcription factorthat has beenactivecan bind toDNAandinducesthe transcriptional activity ofendometriosisresulting inthe synthesis ofmRNAandproteinschange theDNAinto RNAandsynthesis oftarget genesresulting ina majorincrease ininflammatory cytokines(IL-6, IL-8) angiogenesisfactor(HIF -1α, VEGF-A), matrixmetalloproteinase(MMP-2 andMMP-9), anti-apoptotic genes(Bcl-2) anda decrease inpro-apoptotic protein(Bax), increased apoptosisproteins(Caspase 3) andcelladhesion molecules.[14,19]All the factors have a rolein the process ofinvasionanddifferentiation, celladhesionandtissueremodelingthroughoutectopicendometrial stromalcellsof endometriosistosurvive (cell survival)andan increase incell proliferationendometriosis. GenisteinworkedasSERMs, areantiestrogenicinhighestrogen levels. Genisteinbinding affinitytoER-α is 4%, andfor theRE-β was 87%, comparedwith estradiol.[15,18]The difference inthe binding affinityof genistein inER-α andER-β due todifferences inthe aminoacidsequence ofdomainE/Finthe ER-α differs fromregionFER-β thatgenisteintend to have ahigh affinitytoER-β, butalthoughgenisteinhas an affinitywhichis almostequal to the17-β estradiol, inducedstructural transformationofER-β is notenoughto facilitatethe binding ofco-activator in the process ofgene transcription. Estrogen receptorin regulatingtranscription ofgenesalsointeractwithfactorNFkB. Genisteinis aninhibitor ofthe activation ofNFkB(nuclear factor-κB) andSTAT1(signal transducerandactivator oftranscription1) resulting ininhibition ofthe transcription processin protein synthesis

II. Method

This experiment used atrue experimentaldesignweredonein the laboratoryin vivo infemale mice(Musmusculus) withstudy designWithPost-Test Only ControlGroup Design.Involveseightgroups:negative control(healthy micewithoutgivengenistein), positive control group(modelmicegivenendometriosiswithoutgenistein) and thetreatment groupisthe group thatwas givena variety ofdifferent dosesof genistein: 50mg/day, 100mg/day, 200mg/day, 300mg/day, 400mg/dayand500mg/day.

This research was conductedat the Laboratory ofPhysiologyof the Faculty ofMedicine, Universityof BrawijayaandReproductivePhysiology LaboratoryEmbryologiFaculty of Veterinary Medicine, Airlangga University Surabaya. The implementationwas conducted overthreemonths fromAugust toOctober 2014, with detailsfor 1 weekdoneadaptation, 2weeksfortreatment, then usedfor the manufacture ofexaminationpreparationElissathenreadingthe results of research data(statistical test).

Samplesof a studyusingfemale mice(Musmusculus) model ofendometriosisas much as32 head, with2-3 monthsof ageandweigh20-30grams. Musmusculusobtained fromthe Laboratory ofReproductivePhysiologyEmbryologyAirlangga UniversityFaculty of Veterinary Medicine(FKH Airlangga University),Surabaya.Musmusculusselectedas the study samplebecause it is easilymaintained andisrelativelyhealthyanimalsandis suitablefor useinvarioustypesof researchexperimentsandimmunologyresponsescan be observed.

Treatmentdosestoexperimental animals(MusMuculus) will be convertedbythe bodysurface areatothe humanbodyof 70 kgtomice20grams, with aconversionrate0.0026. Micemodel ofendometriosisis based onthe methodperformed onpreliminary researchconducted bySutrisnoetal, 2014.The animalsthat used for experimental werefemale mice(Musmuscullus) approximately 3monthsold, weighing 20-30gramswereselected based oninclusion and exclusion criteria.Afteradaptationinthe same cageandgetthe samefood anddrinkfor 1 week, doreselectionifthere aremicethat qualify asbreaking upthe testornot. Then do theinjection ofcyclosporinAin mice inthe positivecontrol groupandthe treatment group. The drugwhich availableinIndonesiaisSandimmunNovartisproduction.Oneampoulecontains 50mg/mlx 5ml.Thedose is 10mg/kg/day. In this casethe weightof micerange20-30mg, the dose is also adjusted.Afterconversioncalculation at mice and gettinga dose1,8 mg/mice. Sothe doseformice afterreconstitution withwaterforinjection is 0.2ccsandimunafterdiluted.Endometrial biopsymaterialtaken from theuterine operation of benign tumoruterine and storedin PBS.Dowashing2 timeswith acentrifugeat2500rpm. The supernatant wasdiscarded, and thenadded toPBSwithpenicillin200IU/mlandstreptomycin200ug/ml.Eachmousewillget0.1mland theninjected blindto peritonealcavityof micesslowly. Injectionsat intraperitonealendometrial tissueinthe positivecontrol groupandthe treatment group.Performedintramuscularinjection ofestrogenon days1and5.The preparationof ethinylestradiolat a doseof 30µgr/kg. With theconversiontodosethe micewill get5,4µgr. Theequivalent of1 µgr equal with 10 iu. 1 vialcontaining30cccontaining20000 iu, the equivalentof 0.1cc equal with66iu.By adjustingthe doseequivalentconversion mice of5,4µgr equal with 54iu, the micewilleachget arounded0.095ccor0.1cc.Afterinjectingthe micewill be evaluatedwhether theentrycriteria fordroppingthe testor not.Furthermore, afteradaptation, mice weredividedinto 8 groups, one groupas thenegative controlgroup, one groupas thepositive controlgroup, and6 groupsas the treatment group. Genisteinthat has beendissolved insesame oilwill begiven orallybythe sonde. The duration ofgenisteinin the treatment grouprefers to astudy conducted byYavuzetal(2007) on the Granting ofGenisteinonRegressionImplantsEndometriosis inRatModel. Genisteinwas givenfor 14daysand givenonce daily.

Takingmaterialinspection is doneafter 14days oftreatmentwiththe followingsteps: Mice wereterminatedbeforehand byinhalationinanesthetizedby entering the miceintoa coveredcontainer(glass jar), whichcontainscottonthat has beenspilled with ether. Thencover tightlyand wait a fewminutesuntil themicereally did notmove again. Furthermore,mice wereissuedand placedonthe baseboard with thebellyfacing up. Afterpluggingtackson the feetof mice, the abdominal wallwas openedby usingtweezersandscissorscarefully, with amid-lineincisionwas continuedto theleft and right sideonthe top and bottomand thediaphragmis opened.After that, zalirperitoneumis then takenandput ina tubeto be processedin order tomeasurelevels ofhydrogenperoxidein the Laboratoryof Physiology, Faculty ofMedicine, University ofBrawijaya, Malang.

III. Results

In this studythe results ofdata analysis onthe normality testperformed usingthe Shapiro-Wilk test. The criteria forthe decision, that is, whenthe Sigor thep-valueis greaterthanthe significance levelα=0.05 thennormally distributed dataandvice versawhenthe Sigor thep-valueis smallerthanthe significance levelα=0.05 thenthe datawere not normally distributed. In theShapiro-Wilk test analysiswas obtainedanddescribed in detailshown inthe table below.

Table1.Results ofnormality testof data

Groups ofobservations	p-value	distribution

negative controlled	0.453	normal
Positive controlled	0.335	normal
genistein 50 mg/kg weight/day	0.860	normal
genistein 100 mg/kg weight/day	0.448	normal
genistein 200 mg/kg weight/day	0.519	normal
genistein 300 mg/kg weight/day	0.808	normal
genistein 400 mg/kg weight/day	0.603	normal
genistein 500 mg/kg weight/day	0.594	normal

Table 1 based onthe Shapiro-Wilk test resultsshowed thatthe datacontent ofcyclooxygenase-2 (COX-2)(μM) foreach group ofobservationshave demonstratedp-valueof which arelarger thanthe significance levelα=0:05. So allthe datahas met theprerequisitesof parametrictest, the dataproved tobe normally distributed.

In thecomparisonof test resultswith thenegativecontrol groupon the datapositive controlvariablelevelsof cyclooxygenase-2 (COX-2) using independent samplest-test(independent sample t test)are shownbrieflydescribed andas shown inTable2.

Table2.Results ofthe comparisoncontrol group

Variable	**Mean Negative control ± stan.dev**	**Mean positive control±stan.dev**	**p-value**
Levels ofH_2O_2(μM)	0.58±0.09	1.23±0.14	0.000 <∝

Table2 based onthe results ofindependent samplest-test(independent sample t test)showedthatthere were significant differences(p =0.000<α) meanlevels ofcyclooxygenase-2 (COX-2)between thenegative control group(healthy micewithoutgivengenistein) (0.58μM±0:09) withthe positivecontrol group(mice givenmodel ofendometriosiswithout given genistein) (1.23μM±0:14). Based on the main valuelevelsof cyclooxygenase-2 (COX-2) appears inthe negativecontrol groupis smallerin valuewhen comparedwith the average ofthe levels ofcyclooxygenase-2 (COX-2)in thepositive control group.This meansthat themicemodel ofendometriosiswillshow thelevels ofcyclo oxygenase-2 (COX-2)is high when compared tohealthymice.

Based on the resultsof one-wayANOVA teston the datacontent ofcyclooxygenase-2 (COX-2)obtainedsignificant difference inthe meanlevels ofcyclooxygenase-2 (COX-2)sevengroups ofsampleobservations, as shown by thep-value=0.000<α. Furthermore,themultiplecomparison testwith theLeast Significant Differencetest/LSD(Least Significant Difference /LSD) is obtainedand displayedare presentedin Table3.

Table3. Effect ofvariousdosesof genisteinagainst COX-2 inperitonealzalir**(μM))**

Groups ofobservations	**mean ± stan.dev**	**p-value**
positive control	$1.23±0.14^{a}$	0.000<∝
genistein 50 mg/kg weight/day	$1.01±0.10^{b}$	
genistein 100 mg/kg weight/day	$0.89±0.09^{b}$	
genistein 200 mg/kg weight/day	$0.68±0.14^{c}$	
genistein 300 mg/kg weight/day	$0.58±0.08^{cd}$	
genistein 400 mg/kg weight/day	$0.40±0.11^{d}$	
genistein 500 mg/kg weight/day	$0.44±0.11^{d}$	

Table3based onthe results ofthe multiplecomparison testwith LSD testshowedthatthere were significant differencesmeanlevels ofhydrogenperoxide(H_2O_2)between thepositive control group($1.23±0.14^{a}$μM) with theadministration ofgenisteintreatment group50mg($1.01±0.10^{b}$μM), with100mg ofgenistein($0.89±0.09^{b}$μM), with200mg ofgenistein($0.68±0.14^{c}$μM), with300mg ofgenistein($0.58±0.08^{cd}$μM), with400mg ofgenistein($0.40±0.11^{d}$μM), andalsowithgenistein500mg($0.44±0.11^{d}$μM).Based on the mean valuethere is a decreasein the group treatedwith increaseddoses ofgenistein. This meansthat thetreatment ofgenisteinadministration of50mg, 100mg, 200mg, 300mg, 400mg, and500mg in themurinemodel ofendometriosiswill affect thelevels ofcyclooxygenase-2 (COX-2), which isable to reducethe levels ofcyclooxygenase-2 (COX-2) when comparedthemousemodel ofendometriosiswithoutgivinggenistein.Thedifferences between the meanlevels ofcyclooxygenase-2 (COX-2)inthe eighthgroup ofthesampleare presentedin fullappearson the imagehistogrambelow.

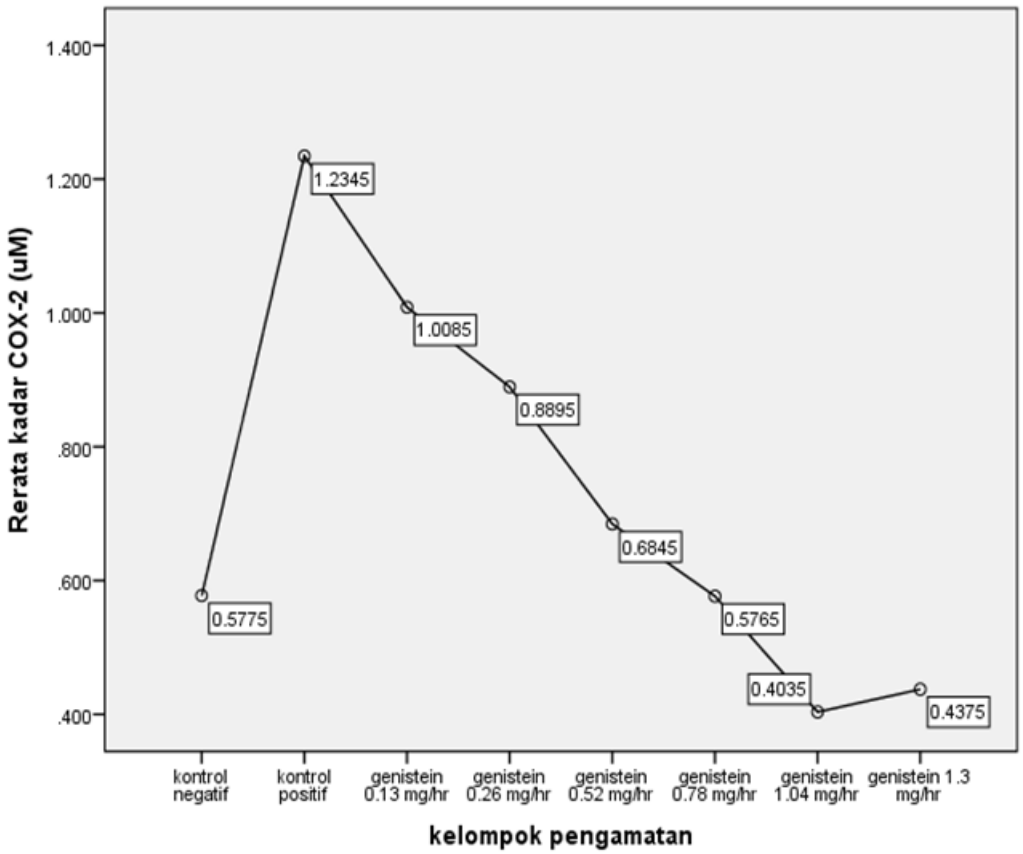

Figure1 Histogrammeanlevels ofCOX-2

In Figure 1 Histogram shows the mean levels of cyclooxygenase-2 (COX-2) in the mouse model of endometriosis at all eighth sample group observations with the administration of genistein treatment dose of 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, and 500 mg. There was an increase in mean levels of cyclooxygenase-2 (COX-2) to the negative control group and positive control group there was a mean decrease in the levels of cyclooxygenase-2 (COX-2) from the positive control group to the treatment group administration of genistein.Looksmeanlevels ofcyclooxygenase-2 (COX-2)decreasedwith increasingdoses ofgenistein. Theaverage value ofthe levels ofcyclooxygenase-2 (COX-2)is the lowestin the group treatedgenisteinadministrationof 400mg. It canbe said thatin this studya dose of 400mg ofgenisteinwereconsidered the mostrapidlyreduce levels ofcyclooxygenase-2 (COX-2)in themousemodel ofendometriosis. Thetrend ofchangebetween groupsobservationsare presentedin Figure2.

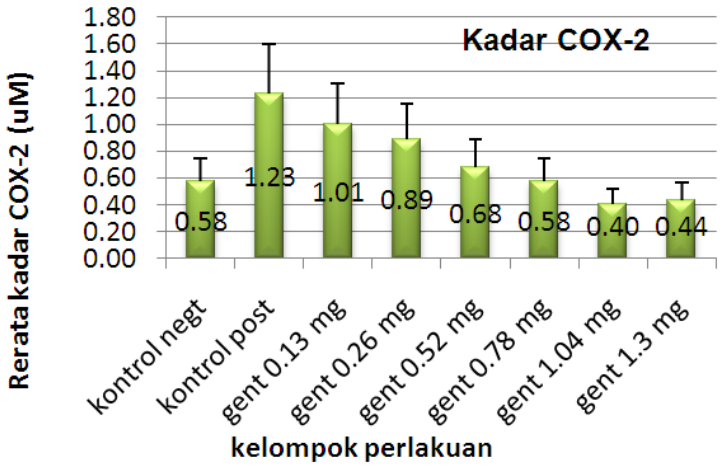

Figure2.Trendschange inmeanlevels of COX-2

Shown inFigure2 showsthe trend ofincrease inthe meanlevels ofCyclooxygenase-2 (COX-2)fromthe negativecontrol groupto thepositive control group.Furthermore, there isa decrease inthe averagelevels ofcyclo oxygenase-2 (COX-2)fromthe positivecontrol groupto thetreatment groupadministrationwith increaseddosesof genistein. Therefore,the average value ofthe levels ofcyclo oxygenase-2 (COX-2) isthe lowestin the group ofgenisteinadministration of400mg sothegenisteindose400mgis a doseof the mostrapidlyreduce levels ofcyclooxygenase-2 (COX-2)dosage-dose compared toothers.

IV. Discussion

There have been manyhypothesesproposedtoexplainthe pathogenesis ofendometriosis, butuntil now nosingle theorythatcanexplain theoverallincidence ofendometriosis. Althoughthe etiology isnot known with certainty, but thejourneyandthe pathogenesis ofthis diseasehasbeen studiedin depthandexpressedina variety oftheoriesrangingfromtheorytobiomolecularclinic. Advances ininvestigating themolecular mechanismsof pathologicalprocessesendometriosis, whichprostaglandinE2(PGE2) plays animportantroleinprosurvivalandimmuneeffects. This isbecause theconcentration ofPGE2was foundinthe peritonealfluidof womenwithendometriosisis muchgreaterthanin theperitonealfluidof womenwith noendometriotsis.[77]

Increased production of prostaglandin E2 100-fold higher in endometriosis due to increased activity of the expression of the enzyme cyclooxygenase-2 (COX-2) induced by IL-1β, TNF-α, MIF and proinflammatory agents. The enzyme cyclooxygenase-2 is the first enzyme involved in converting arachidonic acid (AA) into prostaglandins. The growth of endometriosis involves the role of PGE2. PGE2 is a versatile eicosanoids that have many physiological and pathological functions of a disease. PGE2 involved to play an important role in the development of endometriosis. PGE2 plays a role in regulating the pathophysiological processes including immune suppression, antiapoptosis, angiogenesis and cell proliferation during the development of endometriosis.[75]

GenisteinworkedasSERMs, areantiestrogenicinhighestrogen levels. Genisteinstructurehas similarities-withthe structure of17β-estradiol in the body, it causes genisteinis able tobind tothe ER.[16,18]Genisteinhas an affinityER-β approximately20-30times higherthan theER-α but has aloweractivityof17β-estradiol.[18,19]The highaffinity ofER-β can suppressthe activityofER-α bindsendogenous estrogenby forminga heterodimer. Throughthemechanismof genisteincancompeteoccupyREasREantagonist. Under conditions ofantiestrogenicthenbond withco-regulatory proteinsthat are activatedis the co-repressor, so thatprocessesinhibitedtranscriptionas well asmRNAand protein synthesisresulting inan increase inmajorinflammatory cytokines(IL6, IL8), angiogenesisfactors(HIF-1α, VEGF-A), Matrixmetalloproteinase(MMP-2 andMMP-9), anti-apoptotic genes(Bcl-2) and, increased apoptosisproteins(Caspase 3) andcelladhesion moleculesto be blockedas well aspro-apoptotic proteins(Bax) increases.Genisteinhas a rolein reducinginflammatoryprostanoids, the activity ofCOX-2 andaltercell-signaling. Genisteinrole indownregulationof expressionof COX-2 andNFkBtoreducePGE2.Genisteinprovidesuppressive effecton the production ofarachidonicacid-derived prostanoids, especiallythisPGE2.Prostanoidimplicationsplaysan importantroleinthe immune response againstcancer cells, inflammation, cellproliferation, differentiation, apoptosis, angiogenesis.[42]

GenisteinandDHAcaninhibit the activation ofNFkBbyPPARγ, causingdown-regulation of COX-2 gene, production ofPGE, andsynthesis ofNFkBregulation ofpro-inflammatory cytokines. DHAandgenisteincanalsopressingthe production ofPGE2by changing thefluxthroughthe enzymeCOX-2. In addition, genisteincan potentially interfere withsignal transductioninvolvedin theincreased levels ofcAMP, so as to preventthe effects ofstimulatingthe production ofPGE2onCOX-2 genetranscription.[42] Andthat can be seen from the decreased levels of hydrogen peroxide (H_2O_2) between the positive control group were not given genistein in the treatment group were given genistein.

V. Conclusion

Based onthe explain on the results thehypothesishas beenproven, that the provision ofgenisteincanreduce levels ofCOX-2in thezalir peritoneal of micemodel of endometriosis.

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