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RESEARCH ARTICLE

Antirheumatoid Artritis Effect of Purslane Herb Extract (*portulaca oleracea* l.) to Rat *(rattus norvegicus)* Induced by Complete Freuds Adjuvant

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Abstract

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation of the joints of hands and feet. The research on antirheumatoid arthritis of ethanol extracts from Purslane (*Portulaca oleracea* L.) and effective doses of antirheumatoid arthritis. The research used 15 rats divided into 5 groups. Group 1 (1% Na-CMC); group 2 (prednisone) with the dose of 0.511 mg / kgBW; group III, IV and V were given ethanol extract of purslane doses of 100 mg / KgBW, 200 mg / KgBW and 400 mg / kgWW. The animal model of rheumatoid arthritis was made with intraplant injection of 0.1 mL Complete Freund's Adjuvant. The parameters observed were leg volume measurements and arthritis index. The leg volumes of data were analyzed by One Way Anova test followed by the L test while the arthritis index used the Kruskal-Wallis test followed by the Mann-Whitney test. In leg volume measurements, ethanol extract from the red root dose of 400 mg / kg reduced leg volume by 52.49% ($p < 0.05$). Whereas in the arthritis index parameter, the ethanol extract from purslane dose of 400 mg / kg is significantly reduced the arthritis index ($p < 0.05$) with arthritis index reduction by 50%. In conclusion, the ethanol extract from the purslane (*Portulaca oleracea* L.) with a dose of 400 mg/kg has better activity in treating rheumatoid arthritis in rats.

Keywords: *Antirheumatoid arthritis, Portulaca oleracea L., complete freund's adjuvant.*

Introduction

Rheumatoid arthritis is a chronic inflammatory disease with manifestations of polyartritis, joint destruction and premature mortality. If left untreated, erosion, bone destruction, progressive defects can occur in several months to years and are marked by a relapse period. Pharmacological therapy for rheumatoid arthritis is a non-steroidal anti-inflammatory drug (diclofenac sodium, naproxene and pyroxicam), a drug-modifying antirheumatoid drug class (methotrexate and sulfasalazinne) and corticosteroid drugs (prednisone and methyl prednisolone). However, the use of antirheumatoid arthritis drugs can have considerable side effects [1].

Side effects that can be caused from the use of antirheumatoid arthritis drugs are gastritis, peptic ulcer disease, and kidney disease [2]. Therefore, it is currently developed the use of herbal medicine as an alternative therapy in preventing the development of rheumatoid arthritis. Asmaliani (2018) conclude that methanol extract of jackfruit leaves (*Artocarpus heterophyllus* Lam.) dose of 150 mg/kg body weight have a better activity in treating rheumatoid artritis [3].One of plant that is thought to have activity as antirheumatoid arthritis is purslane (*Portulaca oleracea* L.). It is a plant that can be used as traditional medicine to cure dysentery, skin diseases, and anti-inflammatory [4]. Purslane herb in dose 100 mg/kgBW provide the most effective hypolipidemic activity [5], and in dose 75 mg/kgBW was minimum dose in lowering triglyceride levels [6]. Purslane (*Portulaca oleracea* L.) have a chemical content, namely saponins, flavonoids, omega-3 fatty acids, phenolic acids [7] which are responsible for biological activity.

One of its chemical content, flavonoids, is known to have an anti-inflammatory effect, with the mechanism of inhibiting the cyclooxygenase enzyme so that the formation of prostaglandins is inhibited [8]. Research by Andayani, D, Suprihartini, E & Astuti, M (2018) shows that ethanol extract of purslane (*Portulaca oleracea* L.) at a dose of 100mg /

kgBW, 200mg / kgBW, 400mg / kgBW has an anti-inflammatory effect on mouse leg edema that is induced by caragenine.Inflammation is one of the clinical manifestations of rheumatoid arthritis. Based on the description above, further research is needed on the effects of antirheumatoid arthritis ethanol extract of purslane (*Portulaca oleracea* L.) on rats (Rattus norvegicus) induced by Complete Freund's Adjuvant (CFA). According to Bendele (2011), Complete Freund's Adjuvant (CFA) is an antigen containing heat killed Mycobacterium tuberculosis which can be used as an induction in the occurrence of chronic inflammatory processes.

Materials and Methods

Making Purslane Extracts (*Portulaca oleracea* L.).

Simplicia powder Herbaceous purslane (*Portulaca oleracea* L.) is weighed as much as 500 grams, put in a maceration vessel (jar), then added ethanol 96% until the simplicia powder is all submerged. Left for 3x24 hours, while stirring occasionally at room temperature 20-25 ° C and protected from direct sunlight. After that filtering is carried out and the filtrate is stored in a container vessel, while the residue is soaked again with 96% ethanol. Searching is done twice. The filtrate obtained was then evaporated using a rotary evaporator to obtain a thick extract of Herbaceous purslane (*Portulaca oleracea* L.).

Making 1% b / v Na-CMC [9]

1% b / v Na-CMC suspension was made by weighing 1 gram of Na-CMC then put it in a 100 mL beaker. After that it was dissolved with 50 mL of distilled water which was boiled while stirring using a stirring rod. Then be filled with distilled water up to 100 mL. After cold the beaker is closed using aluminum foil and labeled.

Making Suspension of Extract of Purslane (*Portulaca oleracea* L.)

The suspension of the ethanol extract of Purslane Herb (*Portulaca oleracea* L.) was made by means of dissolving the extract into 1% b / v Na-CMC. The dosage variations that have been made in this study, which are 100 mg / KgBW, 200 mg / KgBW, and 400 mg / KgBW are weighed respectively 100 mg, 200 mg, and 400 mg, then suspended into 10 mL 1% Na-CMC b / v.

Making Prednisone Suspension

Prednisone tablets with a dose of 5 mg were weighed as many as 10 tablets and the average weight was calculated. Tablets are crushed in mortar and weighed equivalent to 0.511 mg of prednisone. Prednisone powder was suspended into 10 mL 1% Na / CMC b / v.

Preparation of Experimental Animals

This study used 15 test animals namely male white rats divided into 5 groups, each group consisted of 3 rats. Before being treated beforehand the test animals are adapted for ± 7 days. to adapt to the new environment, and be fed and drink every day.

Treatment of Experimental Animals [10]

Test animals that have been adapted, then induced with CFA on day 2 and left until the 17th day. On the 17th day grouped randomly into 5 groups, namely the negative control group was given (Na.CMC), the positive contril group was given (prednisone), and the EEHK extract group (ethanol extract of purslane herbs) with successive doses of 100 mg / KgBW , 200 mg / KgBW, 400 mg / KgBW orally for 14 days. Volume foot observation was performed on day 1 before induction with CFA, days 17, -24 and -31 and arthritis index measurements were carried out on days 13, 17, 21, 26 and 31.

Foot Volume Measurement

The effect of antiarthritis drugs was assessed by the percentage inhibition of swelling caused by Complete freund's adjuvant (CFA) which was calculated, in the following way.

% inhibition average =

[(a -b) / a] x 100%

Information :

a = Average volume of rat feet before therapy (Day 17)

b = Average volume of rat feet after therapy (Day 31)

Arthritis Index Measurement [11]

Arthritis index measurements were carried out on days 13, 17, 21, 26 and 31. Arthritis index measurements are carried out based on Table 1.

Table 1: Parameters for Observing the Arthritis Index

Score	Arthritis index parameter score
0	There are no symptoms of arthritis
1	Change in 1 joint
2	Changes in 2 joints
3	Changes in more than 2 joints involved with mild inflammation
4	Changes in more than 2 joints are characterized by polyartritis and ankylosis
5	Very severe polyartritis with ankylosis

In addition, each rat from each group observed an index of the scale of arthritis. Mice were declared rheumatoid arthritis if they had an arthritis index ≥ 2

Discussion

The sample used in this study was ethanol extract of purslane herb (EEPH). Purslane herbs have a chemical content of saponin, flavanoids, omega-3 fatty acids, phenolic acids (Sultana & Rahman 2013, p.38) which are responsible for their biological activities. One of its chemical content, flavonoids, is known to have an anti-inflammatory effect, with its mechanism to inhibit enzymes cyclooxygenase so that the formation of prostaglandin was inhibited. The maceration method is used because the process is simple and can attract compounds that are not resistant to heating [13]. The compounds that are expected to be drawn in this study are flavonoids.

Flavonoids are phenolic compounds that have a conjugated aromatic system. The conjugated aromatic system is easily damaged at high temperatures. Some groups of flavonoids have glycoside bonds with sugar molecules. Glycoside bonds will be easily damaged or broken at high temperatures. This study consisted of two phases, phase 1 (induction phase) and phase 2 (treatment phase). This study began by measuring the initial volume of the left leg of the mouse using the pletismometer on day 1 as the first parameter, after which the induction phase began on day 2. The induction phase was carried out by inducing the left leg of the mouse with CFA suspension (complete freund's adjuvant) as much as 0.1 mL. This induction phase lasts until the 17th day, this is done because according to Woode et al (2008) chronic inflammation begins on the 10th to 28th day which is characterized by the occurrence of polyartritis and edema in the leg area. The mechanism of action of CFA (complete freund's adjuvant) so that it can cause rheumatoid arthritis in mice, namely CFA (complete freunds adjuvant) shows an imbalance in cytokine secretion. The expression of Th2 cytokines such as IL-4 and IL-10 decreased, but Th1 like IFN-α increased. It is thought that Interferon IFN-α stimulates macrophages in local infections and releases inflammatory cytokines such as IL-1 and TNF-α. Tumor necrosis factor (TNF-α) promotes inflammatory cell infiltration, synovium hyperplasia and panus invasion.

IFN-α and TNF-α levels in CFA articular are higher than normal mice and are associated with inflammation [14]. IFN-α and TNF-α levels increase continuously so chronic inflammation is characterized by the occurrence of polyartritis and edema in the leg area. The second phase is the treatment phase, therapy is carried out orally once a day for 14 days, namely on the 18th day to the 31st day. Foot volume observations were carried out on the 17th and 31st days, this was intended to see changes in foot volume due to CFA after induction and administration of the test preparation after therapy. Arthritis index measurements were carried out on days 13, 17, 21, 26 and 31 to observe changes in rheumatoid symptoms during the treatment phase.

Table 2: The average volume of preliminary mice (*Rattus norvegicus*) legs, induction, and after therapy

Group	Average foot volume (mL) ± SD		
	Day-1	Day-17	Day Ke-31
Group I (Na.CMC 1%)	0,37 ± 0,02	0,91 ± 1,09	0,89 ± 0,13
Group II (Prednison)	0,34 ± 0,06	0,90 ± 1,05	0,39 ± 0,06
Group III (EEPH 100 mg/kgBW)	0,34 ± 0,02	0,87 ± 1,01	0,49 ± 0,03
Group IV (EEPH 200 mg/kgBW)	0,38 ± 0,04	0,86 ± 0,96	0,44 ± 0,03
Group V (EEPH 400 mg/kgBW)	0,33 ± 0,94	0,82 ± 0,94	0,40± 0,02

EEPH : Ethanol Extract of Purslane Herb

In table 2, foot volume measurements show that all treatment groups experience rheumatoid arthritis with increased foot volume after CFA induction. This shows that CFA is successful in inducing rheumatoid arthritis in mice. On the 31st day, it can be seen that there was a significant decrease in foot volume in the four treatment groups,

except for the negative control group (Na-CMC). After that, the percentage of the volume of rat feet was calculated which showed the effectiveness of the test group in reducing the volume of rat feet.

Table 3: Percentage reduction in foot volume before and after therapy

Group	Percentage reduction in arthritis index (%)
Group I (Na.CMC 1%)	2,34
Group II (Prednison)	56,73
Group III (EEPH 100 mg/kgBW)	42,80
Group IV (EEPH 200 mg/kgBW)	47,39
Group V (EEPH 400 mg/kgBW)	52,49

EEPH : Ethanol Extract of Purslane Herb

In table 3 shows the percentage data of decreasing foot volume, it is known that the EEPH dose of 400 mg / kgBW has better effectiveness compared to the EEPH dose of 100 mg / kgBW and EEPH 200 mg / kgBW. The reduced volume of activity caused by the activity is almost equivalent to the activity of the positive control group (prednisone). Foot volume reduction data were then analyzed statistically using the One Way Anova test.

The results of One Way Anova analysis showed significantly different results ($p < 0.05$). This shows that there is a difference between groups, then a further analysis is carried out using the LSD Post Hoct test to determine differences between treatment groups. Post Hoct LSD test results showed a positive control group (prednisone) had a value of $p < 0.05$ compared with the negative control group (Na-CMC). This shows that positive control ie prednisone has a significant effect and shows valid data.

For the test group, the group EEPH dose 200 mg / kgBW & EEPH 400 mg / kgBW also had a p value <0.05 compared with the negative control group (Na-CMC). The results of the statistical analysis that has been done, it can be seen that the ethanol extract of purslane herb (*Portulaca oleracea L.*) dose of 200 mg / kgBW and 400 mg / kgBW has an effect as antirheumatoid arthritis, with a p value> 0.05 for positive control (prednisone) and can be seen from the percent decrease in foot volume.

The EEPH group of 400 mg / kgBW had a decreased effect of 52.49% and the EEPH group of 200 mg / kgBW of 47.39 which was not statistically significantly different from the positive control group (prednisone) (p> 0.05). The second test parameter is arthritis scoring. Arthritis scoring was carried out by following the Henson method. This was done by observing the swelling of the four legs of the mice that were scored from 0-5 (Table 1). The severity of arthritis in rat experiments differs according to the individual rats.

Table 4: Average induction of rat arthritis index (*Rattus norvegicus*) induction, and post-therapy

Group	Average induction of rat arthritis index ± SD				
	Day-13	Day-17	Day-21	Day-26	Day-31
Group I (Na.CMC 1%)	3 ± 1	3,67 ±0,58	3,67 ±0,58	3,67 ±0,58	3 ± 0
Group II (Prednison)	3 ± 1	3,33 ±0,58	3 ± 0	2,33 ±0,58	1,33 ± 0,58
Group III (EEPH 100 mg/kgBW)	2,67± 1,53	3,33 ±0,58	3,33 ±0,58	2,67 ±0,58	2,33 ± 0,58
Group IV (EEPH 200 mg/kgBW)	1,67 ± 1,15	2,67 ±0,58	2,67 ±0,58	2,33 ±0,58	1,67 ± 0,58
Group V (EEPH 400 mg/kgBW)	1,33 ± 0,58	3,33 ±0,58	3 ± 1	2 ± 1	1,67 ± 0,58

EEPH : Ethanol Extract of Purslane Herb

In table 4, the observation of arthritis index shows that all treatment groups experienced an increase in volume of feet after CFA induction and decreased leg volume after administration of the test preparation.

From the picture above it is known that all the test groups had activity in reducing swelling in rat feet as seen from the decline in the arthritis index. The arthritis index observation data obtained then calculated the percentage reduction in the arthritis index.

Table 5: Percentage reduction in induction arthritis index and after therapy

Group	Percentage reduction in arthritis index (%)
Group I (Na.CMC 1%)	16,67
Group II (Prednison)	58,33
Group III (EEPH 100 mg/kgBW)	30,55
Group IV (EEPH 200 mg/kgBW)	38,89
Group V (EEPH 400 mg/kgBW)	50,00

EEPH : Ethanol Extract of Purslane Herb

In table 5 shows the percentage data of decreasing therapeutic index, it is known that EEPH 400 mg / kgBW has better activity than EEPH dose of 100 mg / kgBW and EEPH 200 mg / kgBW. The results of the average score of the arthritis index were analyzed statistically using the Kruskal-Wallis showed significantly different results with a significance value of 0.047 ($p < 0.05$). This indicated a difference between groups, to find out the differences between treatment groups, a further analysis was performed using the Mann-Whitney Post Hoct test.

The results of the Mann-Whitney Post Hoct test showed that the positive control group (prednisone) had a p value <0.05 compared to the negative control (Na-CMC). This shows that positive control (prednisone) has a significant effect in decreasing the arthritis index compared to the negative control (Na-CMC). The test group is the group EEPH dose 200 mg / kgBW, and EEPH 400 mg / kgBW also has a p value <0.05 compared with the negative control (Na-CMC).

This shows that the activity of the EEPH dose of 200 mg / kgBW, and EEPH 400 mg / kgBW had a significant effect in decreasing the arthritis index. The results of statistical analysis that have been done on the arthritis index, it can be seen that the ethanol extract of purslane herbs doses of 200 mg / kgBW and 400 mg / kgBW have an effect as antirheumatoid arthritis, as evidenced by the p value <0.05 for the negative group and can also be seen from percentage reduction in arthritis index score. The group dose of EEPH 400 mg / kgBW had a decrease effect of 50.00% and the group EEPH dose of 200 mg / kgBW was 38.89% Based on statistical test analysis, both parameters, namely foot volume measurement and arthritis index observation, found that there were 2 doses, namely purslane ethanol extract at a dose of 200 mg / kgBW and ethanol extract of purslane herbs at a dose of 400 mg / kgBW which has activity in treating rheumatoid arthritis in better mice. However, when viewed from the percentage decrease in the volume of rat feet and arthritis index, it was found that the dose of 400 mg / kgBW had a greater percentage, namely the percentage decrease in foot volume by 52.49% and the reduction in the arthritis index by 50.00%.

This shows that the ethanol extract of purslane herbs at a dose of 400 mg / kgBW is better at treating rheumatoid arthritis in mice than other test doses. This result is similar to the research conducted by Andayani, D, Suprihartini, E & Astuti, M (2018) which states that a dose of 400 mg / kgBW has an anti-inflammatory effect on rat foot edema that is induced by caragenine better than a dose of 100 mg / kgBW and a dose of 200 mg / kgBW.

Conclusion

Based on the results of the research, it can be concluded that:

- Ethanol extract of purslane herb (*Portulaca oleracea* L.) has an effect as antirheumatoid arthritis in male white rats (Rattus norvegicus) induced by Complete Freund's Adjuvant (CFA).
- Ethanol extract of purslane herb (*Portulaca oleracea* L.) dose of 400 mg / kgBW has better effectiveness in treating rheumatoid arthritis in rats (Rattus norvegicus) induced by Complete freund's adjuvant compared to doses of 100 mg / kgBW and 200 mg / kgBW [15, 23].

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