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Randomized controlled trial of herbal extracts *(Eugenia polyantha, Apium graveolens, Nigella sativa)* and allopurinol effect on serum uric acid, urinary uric acid and high sensitivity C-reactive protein levels in subject with hyperuricemia

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ABSTRACT

Background *Eugenia polyantha*, *Apium graveolens*, and *Nigella sativa* are extracts which in preclinical trial can reduce uric acid serum, increase urinary uric acid excretion. Allopurinol is an inhibitor of the xanthine oxidase enzyme which can also reduce the increase of hsCRP in subjetcs with hyperuricemia. **Methods** This study was a double blind randomized controlled trial (RCT). The subjects were hyperuricemic patients aged ≥18 years. The subject was divided into groups that received 3000 mg/day of herbal extracts and allopurinol 100 mg/day for 4 weeks. Evaluation of serum uric acid and urinary uric acid urine were every week, and hsCRP levels was baseline and end of intervention. Other parameters related to the safety of use were examined every 2 weeks. **Results** A number of 44 hyperucemia subjects, 23 subjects received herbal extracts and 21 subjects received allopurinol. The decrease of uric acid serum levels in the herbal extract group was 0,467±1,123;0,600;-2,70-3,00 (p=0.027), while in the allopurinol group 1,114±0,813;1,30;-1,30-2,30 (p=0,000). Uric acid excretion in the herbal extract group decrease 71,00±1,970;5,50;-92,00-702,00 (p=0,269) and in the allopurinol group decrease 64,54±1,298;22,00;-29,00-440,0 (p=0.003). The reduction of hsCRP in the herbal extract group was 0.08±0.639; 0.01; -1.55-2.05 (p=0.658), and the allopurinol group was -0.33 ± 0.806; -0.01; -2.73- 0.31 (p=0.256). **Conclusion** Herbal extracts *(Eugenia poliantha, Apium graveolens* and *Nigella sativa*) and allopurinol can reduce serum uric acid levels in patients with hyperuricemia. Allopurinol also can reduce urinary uric acid excretion.

1. Introduction

Hyperuricemia is a condition in which serum uric acid levels increase ≥ 7 mg / dl in men and ≥ 6 mg / dl in women. It is a risk factor for the onset of arthritis gout, gout or kidney stone nephropathy and the incidence of cardiovascular disease.[1] The prevalence of hyperuricemia as reported in Bali in 2010 about 14.5%.[2] Allopurinol as a widely used as xanthine oxidase inhibitor has reported side effects 5-10%.[3]

Eugenia polyantha, *Apium graveolen*, and *Nigella sativa* are uric acid lowering extracts that have become standardized herbal medicines in Indonesia. In preclinical studies, the composition of

herbal extracts that are effetive at reducing serum uric acid levels is at a dose of 30-40 mg. At a dose of 30 mg it consists of 13 mg (43.3%) bay leaf extract *(Eugenia polyantha)*, 10 mg (33.3%) celery extract *(Apium graveolens)* and 7 mg (23,33%)black cumin seed extract *(Nigella sativa)*.[4] *Eugenia polyantha* contains 0.2% essential oils, flavonoids (quercetin, quercitrin, myricitrin and myricetin) and tannins.[5] Quercitrin and myricetin are the competitive inhibitors of the xanthine oxidase enzyme.[6] The main content of *Apium graveolen* is flavonoids apigenin, inositol, asparagine, glutamine, choline and linamarosa.[5] Flavonoids apigenin is a xanthine oxidase competitive inhibitor enzyme. Infuse 10% of *Apium graveolen* (5 ml/kg) can reduce uric acid levels as same as probenecid 20 mg/kg and associated with increased uric acid excretion. *Nigella sativa* (black cumin) has been shown to increase urine volume and excretion of uric acid in urine in pre-clinical studies.[8,9]

Eugenia polyantha (43.3%), *Apium graveolen* (33.3%) and *Nigella sativa* (23.33%) at doses of 2000 mg/24 hours have been shown to reduce serum uric acid levels by 0,12±0.91 mg/dl ($p < 0.05$) and increase urinary excretion of uric acid 1.78 ± 390.83 mg/24 hours ($p < 0.05$) at day 14th. [10]

C-reactive protein (CRP) is mostly synthesized in the liver. In the transcription phase is affected by proinflammatory cytokines. IL-6 being the main regulator supported by C/EBPβ and C/EBPγ is a key factor of transcription. In addition, IL-6 will provide a signal strengthened by IL-1β and TNFα in enhancing the transcription process of CRP.[11] Increased expression of CRP in conditions of uric acid levels >6mg/dl is caused by activation of p38 and ERK44/42 MAPK.[12] The inflammatory process that occurs is caused by accumulation of monosodium urate (MSU) crystals caused by increasing serum uric acid levels. Monosodium urate binds to caspase I activating NALP3 inflammasone, resulting in the expression of IL-1β and IL-18.[13] In another study it was also mentioned that MSU induces the release of TNF cytokines. Evidence in other studies also states that hyperuricemia is associated with hsCRP levels. [14]

The purpose of this study was to determine the effect of herbal extracts (*Eugenia polyantha, Apium graveolens* and *Nigella sativa*) on serum uric acid, uric acid and high sensitivity c-reactive protein levels in patients with hyperuricemia and the safety of herbal extracts compared with allopurinol.

2.Methods

This study is a double blind randomized controlled trial. Subjects were hyperuricemic patients with inclusion criteria aged> 18 years with blood uric acid levels> 7 mg/dl (male) and > 6 mg/dl (female) and willing to take drugs given with exclusion criteria for liver function disorders with AST >40 U/L; and or ALT >65 U/L; impaired kidney function with creatinine > 1.5 mg/dl or ureum >40 mg/dl; malignancy; use of drugs that affect uric acid levels such as diuretics, pyrazinamides, and salicylic acid; chronic inflammation (RA, IBD); attacks of acute gout; hypersensitivity to allopurinol and use of anti-inflammatory drugs: NSAIDs, steroids and colchicine for the hsCRP variable.

Screening is done to get prospective subjects who meet the inclusion criteria. Subjects who had used gout-lowering drugs, washed out 2 weeks before screening. The samples were randomly divided into 2 groups, group X was the group that received herbal extracts 3 x 2 capsules (@ 500mg) in a day for 28 days, group Y was the group that received allopurinol 3 x 2 capsules (100 mg) in a day for 28 days. Both groups received nutritional education according to the nutrition guide for patients with hyperuricemia from the nutrition department of Kariadi Hospital.

On day 0, day 7, day 14, day 21 and day 28, samples were taken for examination of serum uric acid, urinary uric acid and history and physical examination related to complaint taking the drugs given. On day 0, 14 and 28, a routine blood test was carried out: hemoglobin, leukocytes, platelets,

ureum, creatinine, AST, and ALT. Serum hsCRP levels were taken at day 0 and day 28. Data obtained were recorded in the subject's developmental record.

3.Results

Based on the randomization of subjects divided into two groups, 23 subjects received herbal extracts therapy with a dose of 3 x 1000 mg and 21 people received allopurinol therapy with a dose of 1 x 100 mg allopurinol. The flowchart of the selection of research subjects is presented in **Figure 1** below. Serial analysis (Friedman's test) in both groups showed that there was a significant (significant) decrease in serum uric acid levels 0 to 4 weeks, both in the herbal extracts group (p = 0.026) and allopurinol group (p = 0.000) (Figure 2). The median value for the allopurinol group was lower than herbal extracts starting from the 1st week to the 4th week. In Figure 3 above shows that serial analysis of uric acid excretion in urine in the allopurinol group, there was a significant decrease between week 0 and week 4 (Friedman test, p =0.003), whereas in the herbal extracts group there was no significant difference (Friedman test, p = 0.269). The median uric acid level of the second week in the herbal extracts group was greater than in the allopurinol group, although statistically the difference was not significant (p> 0.05). HsCRP levels in both groups did not differ either at week 0 (p = 0.379), week 4 (p = 0.267), and decrease levels of hsCRP at week 4 from week 0 (p = 0.295). In both groups, there was a decrease in HsCRP level 4 weeks from week 0 (P herbal extract = 0.658 and p allopurinol = 0.256). However, the hsCRP level of the herbal extract group tended to decrease at 4 weeks (0.08 ± 0.639; 0.01; -1.55-2.05) and the allopurinol group tended to increase at the 4th week (-0.33 ± 0.806 ; -0.01; -2,73-0,31).

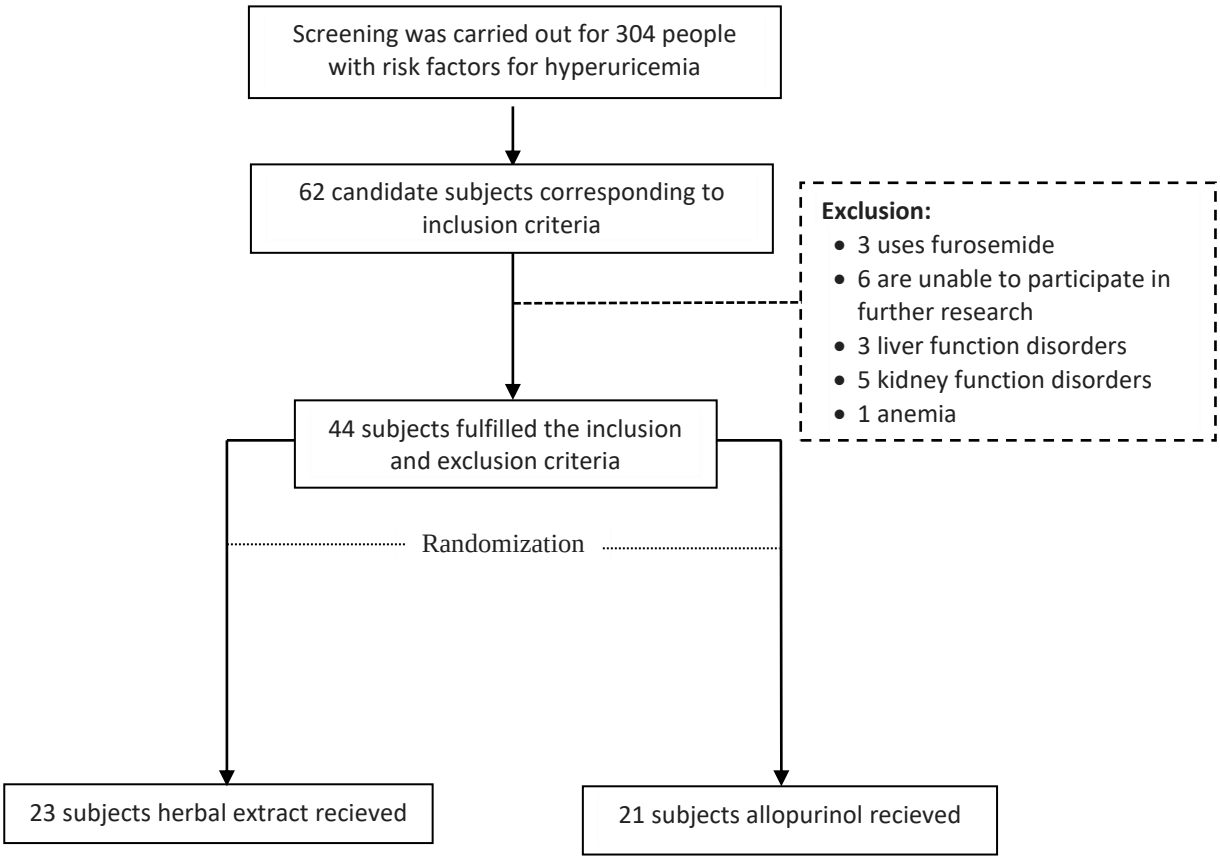

Figure 1. Subjects Disposition

Table 1. Baseline characteristics of Randomized Subjects

Characteristics	**Herbal Extracts (*n*=23)**	**Allopurinol (*n*=21)**	***p***
Age (years old) (mean±SD; median; min-max)	46,8±12,60; 48,0; 26-65	49,5±13,04; 55,0; 26-65	0,439 [a]
Weight (kg) (mean±SD; median; min-max)	73,4±15,83; 71,5; 50-110	74,2±14,89;76,0; 42-99	0,867 [b]
Gender, *n* (%)			
• Male	12 (52,1)	10 (47,6)	1,000 [c]
• Female	11 (47,9)	11 (52,4)	
Obesity, *n* (%)			
• Obesity	14 (60,8%)	14 (66,7)	1,000 [c]
• Non	9 (39,2%)	7 (33,3%)	
Diabetes History, *n* (%)			
• Diabetes	1 (4,4%)	4 (19,0%)	0,267 [c]
• Non Diabetes	22 (95,6%)	17 (81,0%)	
Recent use of Allopurinol, *n* (%)			
• Yes	6 (26,1%)	5 (22,7%)	1,000[c]
• No	17 (73,9%)	17 (77,3%)	
Hypertension, *n* (%)			
• Yes	7 (30,5%)	10 (47,6%)	0,267 [c]
• No	16 (69,5%)	11 (52,4%)	
Hyperuricemia phase *n* (%)			
• Hiperurisemia asimptomatik	12 (52,1%) 11 (47,9%)	9 (40,9%) 13 (59,1%)	0,549 [c]
• Fase interkritikal			

Serum uric acid levels (mg/dl) (mean±SD; median; min-max)	7,59±1,253; 7,25; 6,1-11,7	7,45±1,022; 7,40; 6,1-9,9	0,864[a]
Urinary uric acid levels (mg/dl) (mean±SD; median; min-max)	121,71±1,906; 60,50;5,0-724,0	115,50±1,304; 65,10; 15,0-515,0	0,211[a]
HsCRP leves (mg/dl) (mean±SD; median; min-max)	0,63±0,62; 0,46; 0,08-2,38	0,39±0,31; 0,30; 0,01-3,00	0,379[a]

[a]*Mann-Whitney test* [b] *t-independent test* [c] *Chi-square test*

Table 2. Comparison of Serum Uric Acid Levels in Both Treatment Groups

	Herbal Extracts (*n*=23)	**Allopurinol (*n*=21)**	***p***
Week 0	7,59±1,253; 7,25; 6,1-11,7	7,45±1,022; 7,40; 6,1-9,9	0,864[a]
Week 1	7,51±1,358; 7,05; 6,0-10,5	6,79±1,878; 6,50; 4,4-11,1	0,080[a]
Week 2	7,75±1,258; 6,90; 5,7-10,4	6,60±1,578; 6,60; 4,3-10,4	0,078[a]
Week 3	7,28±1,464; 6,90; 3,9-10,5	6,33±1,444; 6,10; 3,9-10,1	0,040[b]
Week 4	7,13±1,313; 6,95; 4,7-10,5	6,33±1,580; 6,20; 4,0-11,2	0,016[a]

[a]Mann-Whitney test [b] *T*-independent test

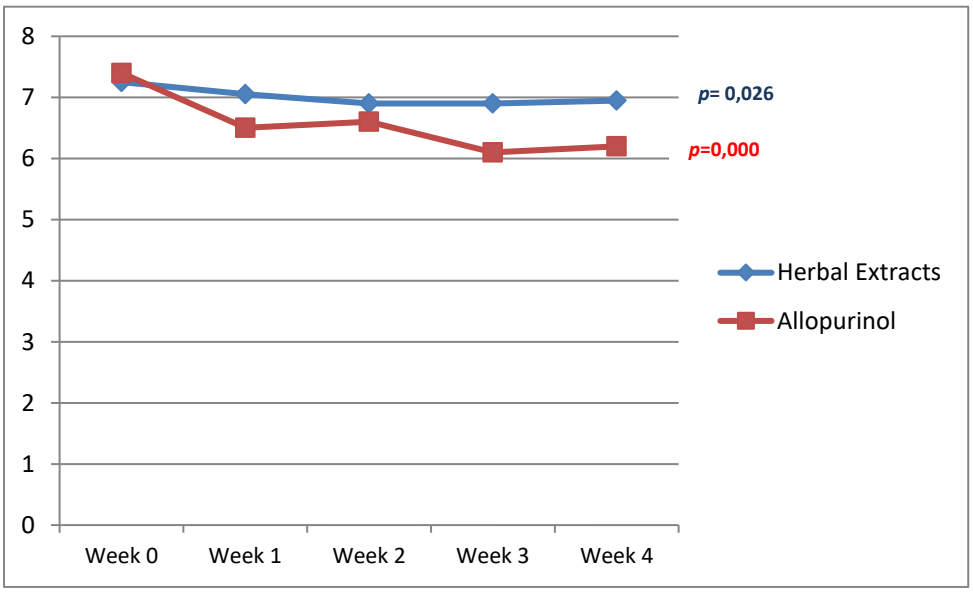

Figure 2. Graphic of Serum Uric Acid Levels in Both Treatment Groups

Table 3. Comparison of Decreased Serum Uric Acid Level in Both Treatment Groups

	Herbal Extracts **(*n*=23)**	Allopurinol **(*n*=21)**	*p*
Week 0 and 1	0,075±0,786; 0,10;-2,75-1,40	0,6619±1,253; 0,90; -3,10-2,00	0,007[a]
Week 0 and 2	0,316±1,220; 0,25; -2,2-4,1	0,847±0,888;1,00;-0,70-2,30	0,032[a]
Week 0 and 3	0,333±1,142;0,40;-2,70-2,70	1,114±0,824;1,142;-0,70-2,40	0,013[b]
Week 0 and 4	0,467±1,123;0,600;-2,70-3,00	1,114±0,813;1,30;-1,30-2,30	0,018[a]

[a]Mann-Whitney test [b] *T-independent* test

Table 5. Comparison of urinary uric acid levels at week 0 to week 4 of the both treatment groups

	Group		p
	Herbal Extracts (*n*=23)	Herbal Extracts (*n*=23)	
Week 0	121,71±1,906; 60,50;5,0-724,0	115,50±1,304; 65,10; 15,0-515,0	0,211[a]
Week 1	71,08±92,273; 52,50; 5,0-481,0	54,50±36,129; 54,0; 7,0-168,0	0,750[a]
Week 2	59,93±30,591; 75,00;13,0-108,0	57,76±30,990; 58,0; 6,0-125,0	0,577[a]
Week 3	58,10±32.109; 49,50; 14,0-113,0	50,28±29,094; 52,00; 7,0-147,0	0,426[a]
Week 4	50,708±31,170; 50,0; 10,0-117,0	50,95±18,540;53,0;11,0-79,0	0,975[b]

[a]Mann-Whitney test [b] *T-independent* test

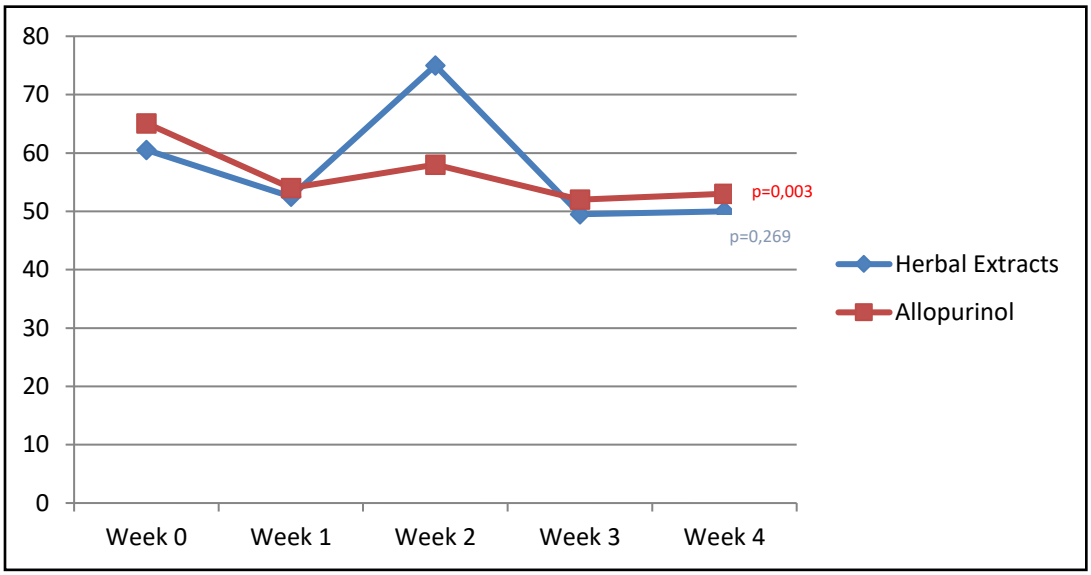

Figure 3. Graphic of Urinary Uric Acid Excretion Levels in Both Treatment Groups

Table 6. Comparison of decrease urinary Uric Acid levels of both treatment groups

	Herbal Extracts (*n*=23)	Allopurinol (*n*=21)	*p*
Week 0 and 1	50,62±2,092 ; 9,0; -407,9-644,0	60,99±1,299;22,00;-44,00-454	0,127
Week 0 and 2	61,77±1,923;-4,65;-54,00-690,00	57,73±1,280;19,00;-38,00-439,00	0,142
Week 0 and 3	63,61±1,827; 0,85; -77,00-623,00	65,21±1,295;32,00;-83,00-455,00	0,054
Week 0 and 4	71,00±1,970;5,50;-92,00-702,00	64,54±1,298;22,00;-29,00-440,0	0,183

Table 7. Comparison of HsCRP levels at week 0 and week 4 of Both Treatment Groups

	Herbal Extracts (*n*=23)	Allopurinol (*n*=21)	*P*
Week 0	0,63±0,62; 0,46; 0,08-2,38	0,39±0,31; 0,30; 0,01-3,00	0,379[a]
Week 4	0,55±0,917;0,70;-1,55-2,050	0,73±0,860;1,40;0,04-3,00	0,267[a]
Decreases levels week 0 and 4	0,08±0,639;0,01;-1,55-2,05	-0,33±0,806;-0,01;-2,73-0,31	0,295[a]
P Week 4th and 0th	0,658[b]	0,256[b]	

[a] Mann-Whitney test [b] Wilcoxon test

4. Discussion

Administration of allopurinol 100 mg/24 hours for 4 weeks found a decrease in serum uric acid levels 1.29 ± 0.629 mg / dl, when compared to the study conducted by Scott et al.(1966) obtained a mean decrease in uric acid levels from 9.3 to 5.8 mg / dl at the same dose as 2 weeks.[15] In another study with 120 days of allopurinol with a mean dose of 261 mg / day it was found to decrease as much as 34% (from 10 to 6.6 mg / dl) .[16]

In the group that received herbal extracts there was a decrease from day 0 to day 28 (week 4) with 0.47 ± 1.123, although compared to the group of allopurinol smaller (1.11 ± 0.813), but the decreased serum uric acid levels with a dose of 3000 mg/day is greater than dose of 2000 mg/day (0.12 ± 0.91). [4]

The Phase III febuxostat study comparing the effectiveness of febuxostat 80 mg/day, 120 mg/day, and 240 mg/day with allopurinol 300 mg/day for 28 weeks with the output of normal serum uric acid levels (< 6 mg/dl) was 76% (122/161), 87% (163/188), and 94% (78/83), while allopurinol 41% (85/208) .[17] In this study the reduction in serum uric acid levels reached target 13% (3/23) for herbal exctract group and 33% (7/21) for allopurinol group (100 mg/day).

The excretion of uric acid in this study was seen that the two groups did not difference. If compared to uric acid excretion at week 0 and week 4, allopurinol decreased uric acid excretion significantly (p = 0.037) with a decrease in uric acid level of urine 24.3 ± 44.11 mg/dl. Previous studies (doses of 2000 mg / day) found a significant increase in the second week (from week 1 (114.11 ± 745.74 mg/day), but after 4 weeks there were no significant differences.[4]

In another study in patients with hyperuricemia who received allopurinol 300 mg for 6 months, there was a decrease in excretion of uric acid levels as much as 36.4%, but decreased slightly when compared with febuxostat 80 mg for 6 months which decreased by 58,6% .[18]

Apium graveolens has uricosuric and diuretic effect. In this study there was no examination of 24-hour urine and only using randomized urine, so diuretic effect of *Apium graveolens* cannot be evaluated, and the synergistic diuretic and uricosuric effects can be considered as reasons for not increasing uric acid levels in the herbal extract treatment group, as well as the effect of xantin oxidase inhibitors that can reduce overproduction of serum uric acid .

Changes in hsCRP levels at week 4 and week 0 in the two groups showed no significant difference, but the herbal extract treatment group experienced a decrease in hsCRP level week 4 from week 0 (0.08 ± 0.639 mg/dl) compared the allopurinol group increased (0.33 ± 0.806 mg/dl).

A study with subject obese postmenopausal women (BMI> 30 kg/m2) received a calorie retention diet and *Nigella sativa* oil 3 grams/day for 8 weeks. Decrease in hsCRP level in the group that received *Nigella sativa* oil more than the group that only received a calorie retention diet (-54.5% compared to -21.4%, with p = 0.01) .[19]

The administration of allopurinol in patients with hyperuricemia with type II DM has been reported to have the effect of reducing hsCRP levels. Allopurinol at a dose of 300 mg for 3 years has been shown to reduce hsCRP levels when compared with the control group (0.16 ± 0.07 mg/dl and 0.35 ± 0.18, with p <0.001) [20]

Allopurinol in patients with hyperuricemia associated endothelial function with hsCRP as a marker is

as done by Kanbay et.al (2011). The subjects of this study were hyperuricemia patients who were treated with allopurinol 300 mg/day for 16 weeks compared to placebo and subjects with normal uric acid levels. In patients with hyperuricemia who received therapy there was a greater decrease in hsCRP (7.4 ± 5.8 mg/dl to 4.6 ± 3.7 mg/dl, p = 0.003) compared with placebo (6.9 ± 3.4 mg/dl to 5.9 ± 3.8 mg/dl, p = 0.04), but it has not been as low as hsCRP level in patients who do not have hyperuricemia (3.4 ± 2.2 mg/dl) .[21]

Some of the studies above were carried out with a duration of 8 weeks of intervention on the use of *Nigella sativa* with a dose of (human) use of 3000 mg/day. Allopurinol in the above study was intervened for a minimum of 16 weeks at a dose of 300 mg/day.

Research Limitations

Some limitations of this study include: intervention duration of 4 weeks, examination of uric acid excretion is measured using random urine, lipid profiles (HDL, LDL and triglycerides) were not examined and no assessment of glycemic status in patients with type II DM.

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