COMBINATION TREATMENT EFFECT OF HABBATUSSAUDA (NIGELLA SATIVA) AND ALLOPURINOL FOR HYPERURICEMIA

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Abstract: *More than 50% of hyperuricemia patients could not maintain a reduction in serum urate at the most commonly used dose of Allopurinol as initial urate lowering drug. Habbatussauda (Nigella sativa) has been shown to increase excretion of urate in pre clinical studies. This research studied the combination effects of Habbatussauda and Allopurinol to induce the effects and to reduce the required high doses of Allopurinol. The design of this study was experimental using a pretest posttest with control group design. This study used 24 mice (Mus muscullus) which were divided into: the negative control group (aquadest), the positive control group that was given Kalium Oxonat as an induction of hyperuricaemia and Allopurinol, the treatment group 1 (Kalium Oxonat and Habbatussauda), and the treatment group 2 (Kalium Oxonat and combination of Allopurinol and Habbatussauda). Statistical analysis was carried out to test the differences in blood urate level before and after treatment, using One-Way ANOVA and Post Hoc Bonferroni. This study found decrease in urate level in the positive control group and all treatment groups which was significantly different between groups ($p < 0,05$). Nevertheless, the reduction in urate level in Allopurinol group was greater insignificantly than in Habbatussauda and Allopurinol combination group ($p > 0,05$). This study concluded that Habbatussauda does not have the potential to increase antihyperuricemia effect of Allopurinol.*

INTRODUCTION

Noncommunicable disease remains a major contributor to mortality throughout the world. Particularly, hypertension and kidney disease are chiefly responsible for this grim reality. Despite recent advances in pharmacotherapy and lifestyle modification, the global prevalence of hypertension and kidney disease still grows at an alarming rate. Interventions including epidemiological and clinical studies indicate that hyperuricemia serves as an aggravating factor in hypertension, eventually leading to kidney disease progression and worsening. Previous studies indicate that hyperuricemia and its association with hypertension could lead to renal vasoconstriction and vascular damage[1]. Hyperuricaemia, the biochemical precursor to gout, has been linked with an increased incidence of, and mortality from, both Congestive Heart Disease (CHD) and stroke[2].

The incidence and prevalence of gout is rising throughout the world. Hyperuricemia prevalence ranged from 2.6 to 47.2%, which varies in different populations[3]. Its pathogenesis is well understood: elevation of serum urate levels above 360 μmol/liter (6 mg/dl) can lead to formation and deposition of monosodium urate crystals in joints and soft tissues that can result in painful acute flares of joint inflammation [4]. Without treatment, flare frequency increases, chronic joint damage occurs, and mobility/function decrease, resulting in impaired health- related quality of life[5]. There is also an increased risk of serious comorbidities (e.g., cardiovascular disease) and premature mortality[2,6].

Hyperuricaemia is associated with an increase in the production of oxygen free radicals, oxidative stress and up-regulation of pro-inflammatory cytokines and mediators[7]. Oxidative stress and reactive oxygen species (ROS) of renal mitochondria have been shown to be associated with hyperuricaemia and hypertension that leads to mitochondrial abnormalities which complicate kidney disease. Uric acid (urate) accumulation in the mitochondria could result from various mechanisms such as Xanthine Oxidase (XO) activity, purine catabolism, and cytoplasmic uptake. Plasma and cellular uric acid levels are tightly regulated by complex processes which include several transporters. Dysregulation of transport activity could potentially lead homeostatic imbalance and uric acid accumulation[1].

Treating gout should be straightforward due to the availability of safe, effective, long-term treatment to lower urate levels (urate- lowering therapy [ULT]), allowing dissolution of existing urate crystals and prevention of new crystal formation, leading to the cessation of gout flares[5]. General practitioners often provide initial management with the use of urate lowering medications such as Allopurinol[8]. Allopurinol, a XO inhibitor, reduces the conversion of hypoxanthine and xanthine to uric acid (uricostatic)[8]. However, Allopurinol can rarely cause Severe Cutaneous Adverse Reactions (SCAR) and Allopurinol Hypesensitivity Syndrome (AHS). Risks of SCAR from allopurinol range from 1:250 to 1:1000, with mortality rates of up to 25%[9,10].

However, clinical trials have shown that more than 50% of patients could not maintain a reduction in serum urate levels at the most commonly used dose of allopurinol (300mg)[11]. In some patients, allopurinol is less effective and induces serious side effects, including pruritus, skin rash, fever, hepatitis, renal toxicity, and leukocytosis with eosinophilia[12]. Thus, it is clinically desirable to combine a compound with allopurinol therapy to potentiate the effects of allopurinol and to reduce the required high doses of the drug for better treatment safety[13]. Other urate lowering medications is uricosuric agents that promote the excretion of uric acid. Uricosuric agents like probenecid and sulfinpyrazone are nephrotoxic. The benzbromarone use is associated with severe fulminant liver toxicity. Thus, the quest for uric acid lowering agent is highly necessary[14].

Habbatussauda (Nigella sativa) has been shown to increase urine volume and excretion of uric acid in urine in pre-clinical studies[15]. Habbatussauda exert relatively important inhibition on XO activities of Xanthine Oxidoreductase (XOR). XOR is one of the important biological sources of ROS and is therefore incriminated in several pathological processes. The enzyme XOR catalyses the oxidation of hypoxanthine and xanthine to uric acid, which plays a crucial role in gout. Thymoquinone in Habbatussauda reduces the levels of carbonyl oxidase product (DNPH) and lipid peroxidase product (4HNE) as markers of oxidative stress in the renal cortex[1]. Thymoquinone also increases natural antioxidant enzyme in the renal[1].

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Phenolic compounds in Nigella sativa are source of flavonoids which possesses excellent antioxidant properties[16]. This research studied the combination effect of Habbatussauda and Allopurinol on uric acid levels. It is hoped that this knowledge can increase the effect of reducing uric acid levels without having to increase the dose of allopurinol, thereby preventing the side effects of allopurinol.

RESEARCH METHOD

The design of this study was experimental using a pretest posttest with control group design. This study used 24 mice *(Mus muscullus)* which were divided into 4 groups: the negative control group that was only given aquadest, the positive control group that was given Kalium Oxonat (250 mg/KgBW) as an induction of hyperuricaemia and anti hyperuricaemia drug Allopurinol (250 mg/KgBW/day), the treatment group 1 was given Kalium Oxonat (250 mg/KgBW) and Habbatussauda (2,6 mg/head/day), and the treatment group 2 was given Kalium Oxonat (250 mg/KgBW) and a combination of Allopurinol (250 mg/KgBW/day) and Habbatussauda (2,6 mg/head/day). The inclusion criteria of mice used in this study were 2-3 months old with a weight of 20-25 g, healthy, active movement, male, not disabled and not used for other studies. The exclusion criteria for mice were sick or dead mice, the blood uric acid level of mice after Kalium Oxonat induced was less than 1,7 mg/dl.

All test animals were adapted for 7 days to reduce stress levels of test animals under laboratory conditions. The animal cages were provided with adequate ventilation and light, then the cages were given in the form of sawdust. Test animals were fasted for 10-12 hours. After that, the test animals in the positive control group and 2 treatment groups were injected intraperitoneally with 250 mg/KgBW of Alloxan Kalium Oxonat to increase uric acid levels. On day 7 after Kalium Oxonat induction, blood uri acid levels were measured in all mice as data on uric acid levels before treatment.

Measurement of blood glucose levels in mice was carried out by holding the tail of the mouse using the right hand. Let the front paws grip the wire covering the cage, then clamp the nape of the mouse with the thumb and forefinger of the left hand. After that, the mouse tail clip from the right hand was transferred to the left hand, then pulled slightly so that the abdomen tensed. The tails of mice were cut (0.2 cm) from the tip of the tail using sterile scissors. The blood that comes out is dripped onto a measuring tube, then the tube is inserted into the spectrophotometer[17].

Habbatussauda (Nigella sativa) used in this study has been identified by the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Riau University. Extract of Habatussauda is made by mashing 1000 grams of Habatussauda in a dry and clean state with a grinder. After that, Habatussauda was soaked using 96% ethanol solvent as much as 5000 mL, then shaken using a mixer for 2-3 hours, then allowed to stand for 24 hours. After that, filtering is carried out which produces the filtrate for later processing in the Rotary Evaporator. When in the Rotary Evaporator, the 96% ethanol solvent is vacuumed, then distilled so that it becomes liquid. The distilled liquid is collected. If all the 96% ethanol solvent has evaporated, then Habbatussauda extract will be obtained. The dose of Habbatussauda given to the 2 treatment groups of mice was 2,6 mg/head/day for 7 days. After 7 days, uric acid levels were measured in all mice as data on uric acid levels after treatment.

RESULTS AND DISCUSSION

This study found decrease in uric acid levels in the positive control group (Allopurinol), treatment group 1 (Habbatussauda) and treatment group 2 (Habbatussauda and Allopurinol) which was significantly different between the three groups (p=0,000). Nevertheless, the reduction in uric acid levels in the control group (Allopurinol) was greater than in treatment group 2 (Habbatussauda and Allopurinol), with no significant difference (p= 0,410). In addition, the reduction in uric acid levels in the positive control group (Allopurinol) was significantly greater (p = 0.03) than in treatment group 1 (Habbatussauda). The results of this research can be seen in table 1.

Table 1. Uric acid levels in groups

Groups	Uric acid levels before treatment	Uric acid levels after treatment	Decreased uric acid levels
Positive Control (Allopurinol)	3,53 ± 0,32	1,06 ± 0,19	2,47 ± 0,19*
1: Habbatussauda	3,10 ± 0,17	1,56 ± 0,08	1,54 ± 0,08
2: Habbatussauda + Allopurinol	3,15 ± 0,27	0,83 ± 0,17	2,32 ± 0,17*

*Not significantly different (p = 0,41

Discussion

This research found a decrease in uric acid levels in the group given Habatussauda to normal uric acid levels. The results of this research are in line with research by Sugiyanto (2019) who also found that Habatussauda can reduce uric acid levels significantly[18]. Thymoquinone in Habbatussauda has the effect of repairing cellular oxidative damage as an antioxidant, by increasing the expression of NrF2 (the nuclear factor erythyroid 2-related factor) and HO-1 (Heme Oxygenase-1) proteins. NrF2 increases and maintains antioxidants and activates HO-1 as an inhibitor of Reactive Oxygen Species (ROS) accumulation which prevents hyperuricemia[1].

Thymoquinone contained in Habbatussauda weakens ROS and suppress hyperuricemia. ROS can pose a risk that is detrimental to cellular integrity and function, resulting in cellular injury such as protein damage and oxidation of enzymes in the body. To combat the negative effects of ROS is to inhibit their formation. Thymoquinone functions to maintain antioxidant enzymes such as glutathione peroxidase, glutathione-S-transferase, catalase which act as nephroprotectives and forms glutathionyl-dihydro-thymoquinone as a strong free radical inhibitor[19].

Habbatussauda also contains zinc. Uric acid, as pro-oxidant in cells, can be associated with zinc, which has the potential to slow down oxidative processes. In biochemical systems, the antioxidant properties of zinc have been well demonstrated. NADPH oxidase is inhibited by zinc, leading to reduced generation of reactive oxygen species (ROS), zinc is able to bind the sulfhydryl groups of various molecules, protecting them from oxidation[20].

The reduction in uric acid levels due to the administration of Habbatussauda was significantly lower (p = 0,003) than the reduction in uric acid levels due to administration of Allopurinol in this research. Allopurinol (4-hydroxy- [3,4d] pyrazolopyrimidine) is the

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current mainstay of urate lowering therapy. It is rapidly and extensively metabolized to an active metabolite, oxypurinol. Its primary action is to reduce the production of urate by competitively inhibiting xanthine oxidase and xanthine dehydrogenase, the enzymes responsible for the conversion of xanthine to urate[21]. The biosynthesis of urate is mediated via xanthine oxidase (XOD) in two stages (conversion of hypoxanthine to xanthine and oxidation of xanthine to urate) of urate generation[22].

The decrease in uric acid levels due to the combination of Habbatussauda and Allopurinol was significantly higher (p = 0,00) than the decrease in uric acid levels due to Habbatussauda in this research. It is possible to use other compounds or natural products to enhance the therapeutic effects of Allopurinol while reducing the drug doses. As a result, the dose-related adverse events of Allopurinol can be reduced[13]. Allopurinol significantly normalized, and reversed, changes in the measured serum levels of antioxidants and the lipid peroxidation, thus suggesting that Allopurinol increased antioxidant enzyme activities through their impact on oxidative stress[7]. Allopurinol induces up-regulation in Organic Anion Transporter (OAT1 and OAT3), alongside down-regulation in Urate Anion exchanger Transporter 1 (URAT1) in the kidneys, thus indicating an enhancement of urate excretion reducing serum Uric Acid levels[7].

Nevertheless, in this research the reduction in urate level of Allopurinol was greater insignificantly than in Habbatussauda and Allopurinol combination (p > 0,05). The combined administration of Allopurinol and nature products necessarily down-regulate URAT1 and up-regulate OAT1[13]. Apart from that, the effect of reducing uric acid is also supported by Habbatussauda which prevents the loss of antioxidant enzyme activities such as aconitase and superoxide dismutase. Those enzymes are inhibited directly or indirectly associated with hyperuricemia[1]. The combination treatment of Allopurinol and nature products should reduce Xanthine Oxidase that prevents the formation of uric acid and reduces the levels of serum uric acid by decreasing purine synthesis[13].

CONCLUSIONS

The reduction in uric acid (urate) due to the combination of Habbatussauda and Allopurinol is greater than the reduction in uric acid levels due to Habbatussauda. The urate reduction of combination use of Habbatussauda and Allopurinol is not better than urate reduction of Allopurinol alone. Therefore, Habbatussauda does not have the potential to increase antihyperuricemia effect of Allopurinol.

Additional researches are needed at the future to study other antyhiperuricemia effect indicators of Habbatussauda and Allopurinol combination. Those are level of Xantine Oxidase, transporters associated with urate excretion and antioxidant enzyme activities.

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