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XANTHINE OXYDASE INHIBITION OF *KOMBUCHA TEA* IN HYPERURICEMIA INDUCED WISTAR RAT: decrease of uric acid, malondialdehyde, and 8-hydroxy-2'-deoxyguanosine

I Dewa Made Sukrama
Faculty of Medicine Udayana University, Bali-Indonesia

Background: Hyperuricemia is a condition of high level of uric acid in the body due to distortion of purine nucleoside metabolism through hipoxanthin, xanthin, and guanin of basic purine. **Objective:** to find a cure of hyperuricemia base on the utilization of *kombucha tea*. **Methods:** This is a true experimental study by applying posttest only control group design to determine whether kombucha tea inhibit xanthine oxidase in hyperuricemic induced rat reveales by decrease of uric acid, malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine **(**8-OHdG). In this study, hyperuricemia rat was achieved by intake of high purine diet. Rats were fed with a mixture of 4 g/kg BW of *Gnetum gnemon* with 50 mL/kg BW of chicken liver *ad libitum* for 9 days. Treatments in this research are combination of fermentation time of Kombucha tea and volume of this tea, i.e fermentation time 4, 8, and 12 days and the volume are 1 mL and 4 mL. Therefore, there would be seven groups of treatment including control group. ANOVA was then applied to determine the treatment effect with $p < 0.05$ was concidered significant. **Results:** This study indicates that kombucha tea has an ability to inhibit xanthine oxidase in hyperuricemic induced rat and decrease uric acid, MDA, and 8-OHdG. This ability was achieved with combination treatment of 12 days fermentation and 4 mL of kombucha intake. Xanthine oxidase, uric acid, MDA, and 8-OHdG levels by this treatment were obtained significantly lower compare to control group. **Conclusion:** This study proved that kombucha tea was potent to cure hyperuricemia of wistar rat via inhibition of xanthine oxidase produced.

Keywords: hyperuricemia; rat; kombucha tea; xanthine; oxidase.

INTRODUCTION

Uric acid is a metabolic product of exogenous (brought in with food) or endogenous purine bases. This acid in most physiologic fluids is an end product of purine degradation. The serum urate level in a given patient is determined by the amount of purines synthesized and ingested, the amount of urate produced from purines, and the amount of uric acid excreted by the kidney (and, to a lesser degree, from the gastrointestinal tract).[1,2] Gout is an inflammatory arthritis caused by the deposition of monosodium urate crystals in tissues.[1] This condition typically occurs after years of sustained hyperuricemia. It is estimated to affect 5.1 million people in the United States according to the most recent National Health and Nutrition Examination Survey (NHANES III).[2] Gout affects approximately 2% of men older than 30 years and 2% of women older than 50 years, and is the most common form of inflammatory joint disease in men older than 40 years. Serum uric levels are, on average, 0.5 to 1.0 mg/dl higher in men than women, making male sex a risk factor for hyperuricemia and gout. Lower serum uric levels in women are associated with the presence of estrogen, which is thought to act as an antihyperuricemic.[3] In Indonesia, based on Health Survey in the year of 2005, there were around 10-20% men and post menopause women have a higher levels of uric acids than normal person.[4] It was proven that, celery seed is often used in treating this condition, as it possesses many anti-inflammatory compounds. Other helpful herbs include turmeric, boswellia, cayenne, colchicum and hyssop were also potent to treat hyperuricemia. Clearly, uric acid is produced by purine nucleoside metabolism through hipoxanthin, xanthin, and guanin basic purine. Distortion of this metabolism leads to elevate level of uric acid and known as hyperuricemia.[5] *Kombucha tea* is a traditional fermented tea, empirically in Balinese traditional medicine was proven as a cure of hyperuricemia. This study was carried in order to investigate the ability of *kombucha tea* to inhibit further uric acid formation in the hyperuricemia wistar rat.

Address of Correspondence: I D. M. Sukrama
Faculty of Medicine Udayana University
Bali-Indonesia
Email: dewa_sukrama@yahoo.co.id

METHODS

This is an experimental study with posttest only control group design to observe inhibition activity of kombucha tea in hyperuricemia induced rat. A number of 28 wistar rats were employed in

this study and divided into 7 groups, i.e. control group (C), the first group (T1) treated with 1 ml of 4 days fermented kombucha tea, the second group (T2) treated with 1 ml of 8 days fermented kombucha tea, the third group (T3) treated with 1 ml of 12 days fermented kombucha tea, the fourth group (T4) treated with 4 ml of 4 days fermented kombucha tea, the fifth group (T5) treated with 4 ml of 8 days fermented kombucha tea, and the sixth group (T6) treated with 4 ml of 12 days fermented kombucha tea. Before treatment rats were induced to hyperuricemia by feeding with a mixture of 4 g/kg BW of *Gnetum gnemon* with 50 mL/kg BW of chicken liver *ad libitum* for 9 days.

Animal ethical clearance was obtained from a local authority body at Veterinary Faculty Udayana University, Bali-Indonesia. Around 1 mL of blood was taken from rat heart aorta which was anesthesia before proceeding. The blood was then centrifuged for 15 minutes at the rate of 3.000-3.500 rpm. Uric acid reagent, FS TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid) was then added to the serum obtained. The mixture was then incubated for 10 minutes at a temperature of 37^0C. Then, optical density of the mixture was determined using sphectrophotometer at 546 nm of wave number.

Anova was performed to determine the different effect amongst treatment with p<0.05 was considered significant.

RESULTS

Decrease of uric acid of hyperurisemic rat

Based line data of uric acid of hyperurisemia rat were presented in Table 1.

Data of uric acid decrease of hyperurisemic rat were presented in Table 2. Anova one way was then applied to determine the treatment different. The data were presented in Table 3

Table 1
Uric Acid Levels of Hyperurisemia Wistar Rat

No	Group	Uric acid (mg/dL)			
		Day-6th	*p**	Day-9th	*p**
1	C	2.63±0.10	0.688	3.79±0.19	0.063
2	T1	2.76±0.07	0.584	4.06±0.86	0.842
3	T2	2.72±0.04	0.714	4.11±0.83	0.751
4	T3	2.60±0.06	0.473	3.59±0.59	0.092
5	T4	2.70±0.05	0.329	3.93±0.91	0.322
6	T5	2.94±0.54	0.067	4.10±0.11	0.102
7	T6	3.17±0.58	0.070	4.11±0.09	0.068
	*p***	0.083		0.064	

C = control, T = treatment, *p** significance for normality data (normally distributed at $p>0.05$). *p*** significance for homogenous of variance (homogenous at $p>0.05$)

Decrease of xanthine oxidase on hyperurisemic rat

Xanthine oxidase ativity data were determined as IC_{50} (*inhibitory concentration*) and presented in Table 4. One way Anova was employed to determine the difference of xanthine oxydase activity among treatment group and control group. Resume of the analysis was presented in Table 5.

Table 2
Average of Uric Acid Concentrattion on Hyperurisemic Rat On day-14th and day-19th after treatment

No.	Groups	Uric acid (mg/dL)			
		Day-14th	*p**	Day-19th	*p**
1	C	4.85±0.69	0.411	5.74±0.56	0.536
2	T1	3.86±0.94	0.526	3.64±0.70	0.487
3	T2	4.11±0.83	0.756	3.98±0.88	0.855
4	T3	3.52±0.56	0.075	3.37±0.52	0.445
5	T4	3.87±0.89	0.310	3.71±1.01	0.527
6	T5	3.91±0.31	0.076	3.48±0.27	0.125
7	T6	4.06±0.09	0.149	2.08±0.18	0.396
	*p***	0.137		0,068	

*p** significance for normality data (normal at $p > 0.05$).*p*** significance for homogenous of variance (homogenous at $p > 0.05$)

Tabel 3
Resume of Anova One Way of Uric Acid of hyperurisemic rat on day-14th and day-19th after treatment

Groups		Different of uric acid levels (mg/dL)			
		Day-14th	*p**	Day-19th	*p**
C	T1	0.99	0.053	2.10	0.001
	T2	0.74	0.143	1.76	0.001
	T3	1.32	0.012	2.37	0.001
	T4	0.98	0.057	2.03	0.001
	T5	0.94	0.066	2.26	0.001
	T6	0.78	0.120	3.66	0.001
T1	T2	0.26	0.603	0.33	0.479
	T3	0.33	0.499	0.28	0.557
	T4	0.02	0.976	0.07	0.881
	T5	0.05	0.915	0.16	0.728
	T6	0.21	0.672	1.57	0.003
T2	T3	0.59	0.237	0.61	0.202
	T4	0.24	0.625	0.26	0.575
	T5	0.20	0.679	0.49	0.295
	T6	0.48	0.923	1.90	0.001
T3	T4	0.35	0.480	0.35	0.462
	T5	0.39	0.434	0.11	0.810
	T6	0.54	0.276	1.29	0.011
T4	T5	0.04	0.939	0.23	0.619
	T6	0.19	0.694	1.64	0.002
T5	T6	0.16	0.752	1.41	0.006

C = control, T = treatment, *p** significance at $p < 0.05$.

Decrease of MDA Plasma of Hyperurisemic Rat

Data of MDA levels of hyperurisemis rat were presented in Table 6. Then, one way Anova was employed to determine the different of MDA decrease among control and treatment groups. Resume of the one way Anova was presented in Table 7.

Decrease of 8-OHdG serum of Hyperurisemic Rat

Data of 8-OHdG levels of hyperurisemic rat were presented in Table 8. One way Anova was

then applied to determine the difference between 8-OHdG among control and treatment groups. Resume of the results were presented in Table 9.

Table 4
IC_{50} of Xanthin Oxydase in Hyperuricemic Rat At day-14th and Day-19th after treatment

No.	Group	Xanthine Oxydase (ng/dL)			
		Day-14th	p*	Day-19th	p*
1	C	55.98±1.95	0.334	56.24±1.25	0.572
2	T1	54.12±0.69	0.489	53.62±0.65	0.438
3	T2	54.08±0.85	0.761	53.97±0.82	0.859
4	T3	53.52±0.56	0.475	53.37±0.52	0.445
5	T4	53.87±0.89	0.310	53.71±1.01	0.527
6	T5	53.91±0.31	0.403	53.48±0.27	0.125
7	T6	54.09±0.58	0.059	42.63±0.55	0.068
	p**	0.157		0.278	

C = control, T = treatment, *p** significance for normality data (normal at $p > 0.05$). *p*** significance for homogenous of variance (homogenous at $p > 0.05$)

Table 5
Resume of One Way Anova of IC_{50} Xanthine oxydase on day-14th and day-19th

Groups		Different of IC_{50} XOD (mg/dL)			
		Day-14th	p*	Day-19th	p*
C	T1	1.86*	0.013	2.62*	0.001
	T2	1.91*	0.011	2.27*	0.001
	T3	2.46*	0.002	2.87*	0.001
	T4	2.11*	0.006	2.52*	0.001
	T5	2.07*	0.006	2.76*	0.001
	T6	1.88*	0.012	13.60*	0.001
T1	T2	0.04	0.951	0.36	0.534
	T3	0.59	0.394	0.25	0.661
	T4	0.25	0.721	0.10	0.867
	T5	0.21	0.762	0.14	0.809
	T6	0.02	0.977	10.98*	0.001
T2	T3	0.55	0.428	0.61	0.294
	T4	0.21	0.767	0.26	0.648
	T5	0.17	0.684	0.49	0.391
	T6	0.02	0.684	11.34*	0.001
T3	T4	0.35	0.617	0.35	0.546
	T5	0.39	0.580	0.11	0.843
	T6	0.58	0.410	10.73*	0.000
T4	T5	0.04	0.957	0.23	0.683
	T6	0.23	0.743	11.08*	0.001
T5	T6	0.19	0.784	10.84*	0.001

C = control, T = treatment, *p** significance at $p < 0.05$.

Tabel 6
MDA of Hyperurisemic Rat at Day-14th and Day-19th after treatment

No.	Groups	MDA plasma (µM/L)			
		Day-14th	p*	Day-19th	p*
1	C	1.68±0.02	0.755	1.68±0.02	0.783
2	T1	1.66±0.04	0.764	1.66±0.02	0.577
3	T2	1.65±0.04	0.306	1.62±0.02	0.177
4	T3	1.63±0.06	0.159	1.60±0.02	0.880
5	T4	1.63±0.04	0.492	1.58±0.02	0.572
6	T5	1.59±0.06	0.671	1.56±0.04	0.192
7	T6	1.36±0.08	0.145	1.08±0.11	0.152
	p**	0.488		0.118	

C = control, T= treatment, *p** significance of normality data (normal at $p > 0.05$). *p*** significance for homogeneous of variance (homogenous at $p > 0.05$)

Table 7
Resume of One Way Anova of MDA at Day-14th and Day-19th after treatment

Groups		Difference of MDA (µM/L)			
		Day-14th	p*	Day-19th	p*
C	T1	0.02	0.670	0.02	0.573
	T2	0.03	0.415	0.06	0.107
	T3	0.05	0.220	0.07*	0.047
	T4	0.04	0.257	0.09*	0.011
	T5	0.09*	0.024	0.12*	0.002
	T6	0.34*	0.001	0.58*	0.001
T1	T2	0.01	0.694	0.05	0.139
	T3	0.03	0.415	0.04	0.280
	T4	0.03	0.472	0.08*	0.038
	T5	0.07	0.059	0.10*	0.007
	T6	0.33*	0.001	0.57*	0.000
T2	T3	0.02	0.670	0.01	0.672
	T4	0.01	0.743	0.02	0.504
	T5	0.06	0.125	0.05	0.167
	T6	0.31*	0.001	0.52*	0.001
T3	T4	0.01	0.921	0.04	0.280
	T5	0.04	0.257	0.06	0.077
	T6	0.29*	0.001	0.53*	0.001
T4	T5	0.05	0.220	0.03	0.461
	T6	0.29*	0.001	0.49*	0.001
T5	T6	0.25*	0.001	0.47*	0.001

C = control, T= treatment, *p** significance at $p < 0.05$.

Table 8
Data of 8-OHdG of Hyperurisemia Rat At Day-14th and Day-19th after treatment

No.	Groups	8-OHdG (ng/mL)			
		Day-14th	p*	Day-19th	p*
1	C	0.62±0.03	0.240	0.61±0.03	0.238
2	T1	0.64±0.01	0.683	0.63±0.01	0.272
3	T2	0.65±0.01	0.124	0.61±0.01	0.406
4	T3	0.63±0.03	0.734	0.61±0.01	0.161
5	T4	0.61±0.02	0.239	0.59±0.01	0.124
6	T5	0.58±0.01	0.406	0.51±0.01	0.972
7	T6	0.56±0.01	0.161	0.39±0.03	0.962
	p**	0.135		0.106	

C = control, T = treatment, *p** significance for normality data (normal at $p > 0.05$). *p*** significance for homogenous of variance (homogenous at $p > 0.05$)

DISCUSIONS

This research indicated that there was decrease of uric acid levels of hyperurisemic rat after intake of *kombucha tea*. Treatment with 4 mL of 12 days fermented kombucha gave the significance highest decrease of uric acid. The decrease was 3.66 mg/dL ($p < 0.05$) compare to control.

Setiawan and Suyono (2012) in their study obtained that there was also decrease of uric acid of hyperurisemic rat treated with kombucha. They obtained that the highest decrease was 54% with a dose of 8mL/d of 14 days fermented kombucha.

The advantages of kombucha have already been reported widely. The target is not a certain organ, instead of affecting all system of metabolism in the body and detoxification. This will leads to increase of endogenous immune system towards any penetration of xenobiotic. However,

mechanism of how its work has not been understood completely.

Table 9
One Way Anove of 8-OHdG of Hyperurisemic Rat at Day-14th and Day-19th after treatment

Groups		Difference of 8-OHdG (ng/mL)			
		Day-14th	*p**	Day-19th	*p**
C	T1	0.03	0.080	0.02	0.114
	T2	0.03*	0.026	0.01	0.856
	T3	0.02	0.282	0.01	1.000
	T4	0.01	0.470	0.02	0.114
	T5	0.04*	0.012	0.08*	0.001
	T6	0.06*	0.001	0.22*	0.001
T1	T2	0.01	0.587	0.02	0.158
	T3	0.01	0.470	0.02	0.114
	T4	0.04*	0.018	0.05*	0.003
	T5	0.06*	0.001	0.09*	0.001
	T6	0.08*	0.001	0.24*	0.001
T2	T3	0.02	0.212	0.01	0.856
	T4	0.04*	0.005	0.03	0.081
	T5	0.07*	0.001	0.08*	0.001
	T6	0.09*	0.001	0.22*	0.001
T3	T4	0.03	0.080	0.02	0.114
	T5	0.05*	0.001	0.08*	0.001
	T6	0.07*	0.001	0.22*	0.001
T4	T5	0.03	0.056	0.05*	0.001
	T6	0.05*	0.003	0.19*	0.001
T5	T6	0.02	0.212	0.14*	0.001

C = control, T = treatment, *p** significane at $p < 0.05$.

In this study, it was obtained that kombucha tea decrease uric acid levels of hyperurisemic rat. The uric acid levels decrease was followed by decrease of xanthine oxydase. As indicates in Table 4, treatment of 4 ml of 12 days fermented kombucha results the highest decrease, 2.76 mg/dl compare to control and others treatments. Therefore, it can be stated that the role of kombucha tea in decreasing uric acid in hyperurisemic rat is as an inhibitor the formation of xanthine oxydase.

It was known that uric acid is produced during catabolic of nucleotide purine through a process catalyzed by xanthine oxyreductase (XOR) in the liver process leads to hypoxanthine oxidation to form xanthine and further oxidation forming uric acid.[12] During formation of uric acid, reactive oxygen species was also produced which was significantly increase vascular oxidative stress.[12]

XOR is an hepar enzyme that catalyze uric acid, nitrous oxide, and reactive oxygen species which were potent to damage deoxyribonucleic acid, ribonucleic acid and its protein, inactivated enzymes, amino acids oxidation, and peroxydation of lipids.[13] XOR can be present in two inter-convertible form, i.e. XO-xanthine oxydase and XDH-xhantine dehidrogenase.[14]

Increase of lipids peroxydation leads to increase of free radical results in increase of malondialdehyde formation as a marker of free radical on blood. Increase of uric acid production has also related to increase the formation of reactive oxygen species and also increase of lipid peroxidation. Lipid peroxidation produces MDA as a marker of damaging membrane cell. In this study, we observed the highest significant decrease of MDA on treatment of 4 ml of 12 days fermented kombucha tea. This also consistence to the data obtained for uric acid and xanthine oxydase decrease. In line with this finding, we also obtained that there was a significant decrease of 8-OHdG, a marker for DNA damage. The highest decrease was also gained for treatment of 4 ml of 12 days fermented kombucha tea. Increase of xanthine oxydase on myocardial patients will also be followed by increase of MDA. Since, hyperuricemia can also lead to myocardial destruction, therefore, this situation probably happen in hyperuricemia patients.[13]

Deoxyguanosine (dG) is one of DNA component and during oxidation form 8-hydroxy-2'-deoxyguanosine (8-OHdG). Therefore, 8-OHdG is a product of DNA oxidation cause by ROS. Guanosine hydroxilation is a response of normal metabolic process or can be due to other exogenous factors. Not too many research regardless of the role of kombucha tea in reducing increase of 8-OHdG. The role of green tea in inhibition of DNA damage was known through 8-OHdG increase on mice expose with tobacco. They obtained that obtained there was an inhibition of increase of 8-OHdG.[7]

CONCLUSION

Role of *kombucha tea* in decreasing of uric acid in hyperuricemia rat is through inhibition of xanthine oxydase formation. This was proven by decrease of uric acid which was followed by decrease of xanthine oxydase.

After exposure phase, since *kombucha tea* is a polar matter, no metabolism of phase-I occurs. The tea will be absorbed straight forward and distributed along with blood stream.

Pharmacodynamic phase occurs with formation of ligand in the hepar and followed by decrease of xanthine oxydase formation.

Kombucha tea was also potent to decrease MDA, a product of membrane cell damage due to ROS which was formed during the production of uric acid.

In this research, we also observed that there was a decrease of 8-OHdG as a marker of DNA damage.

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