POTENSI ANTIOKSIDAN DALAM EKSTRAK TEH MERAH (*Hibiscus sabdariffa)* DAN TEH HIJAU (*Camellia sinensis*) TERHADAP PROSES ATEROGENESIS PADA TIKUS DENGAN DIET ATEROGENIK

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RINGKASAN

Teh merupakan minuman yang secara universal dikonsumsi di banyak negara serta di berbagai lapisan masyarakat. Teh menempati peringkat kedua dikonsumsi oleh masyarakat sedunia setelah air. Beberapa studi epidemiologis mengindikasikan bahwa konsumsi polifenol teh hijau maupun polifenol herbal lainnya dapat menurunkan angka kejadian penyakit degeneratif khususnya resiko aterosklerosis. Perlu diteliti lebih lanjut potensi dan mekanisme antioksidan pada ekstrak teh merah (*Hibiscus sabdariffa*) dan teh hijau (*Camellia sinensis*) terhadap pencegahan proses aterogenesis dan stres oksidatif pada tikus yang mengkonsumsi diet aterogenik.

Melihat potensi teh hijau maupun teh merah sebagai *functional food* penulis ingin meneliti lebih lanjut potensi dan mekanisme antioksidan pada ekstrak teh merah (*Hibiscus sabdariffa*) dan teh hijau (*Camellia sinensis*) terhadap proses aterogenesis dan stres oksidatif endotel. Bila hal ini dapat dibuktikan dan dapat dijelaskan maka diharapkan dapat menurunkan resiko aterosklerosis yang akhirnya menurunkan resiko penyakit kardiovaskular. Pertimbangan lain, penulis melihat baik teh merah maupun teh hijau memiliki "*soft image*" bagi orang yang sehat maupun yang memiliki kondisi sindroma metabolik dan beresiko terhadap penyakit jantung koroner, karena komoditi ini bukan obat tetapi lebih dianggap sebagai *daily drink* atau *regular drink* yang natural dengan spektrum konsumen yang luas, efek samping dan reaksi alergi yang minimal sehingga diharapkan lebih mempermudah untuk terapi penurunan resiko penyakit degeneratif.

Tujuan penelitian ini adalah untuk menjelaskan efek preventif pemberian ekstrak teh merah (*Hibiscus sabdariffa*) dan teh hijau (*Camellia sinensis*) terhadap keadaan stress oksidatif dan aterosklerosis pada tikus yang diberi diet aterogenik. Penelitian ini merupakan penelitian eksperimental laboratoris, dimana baik sampel (hewan coba) maupun perlakuan lebih terkendali, terukur dan pengaruh perlakuan dapat lebih dipercaya. Rancangan penelitian menggunakan *The Randomize separate sample pretest-post-test control group design* (Campbell & Stanley, 1966), karena pada setiap pengambilan unit analisis, yaitu darah dan jaringan aorta hewan coba harus dikorbankan (dibunuh), sehingga tidak memungkinkan menggunakan hewan coba yang sama untuk mendapatkan data pretest dan post-test. Pembagian hewan coba ke dalam kelompok dilakukan dengan cara random, sehingga semua semua hewan coba memiliki kesempatan yang sama untuk menempati semua kelompok. Hal ini bertujuan untuk meningkatkan validitas internal.

Hewan coba yang digunakan dalam penelitian ini adalah tikus putih jenis wistar (*Rattus norvegicus strain wistar*) jantan, umur ± 2 bulan, dimana hewan ini merupakan mamalia yang fungsi fisiologis organ tubuhnya hampir sama dengan manusia, murah dan mudah pemeliharaannya.

Pakan yang diberikan pada tikus selama penelitian ada 2 (dua) macam yaitu diet standart atau normal dan diet aterogenik. Diet standart atau normal adalah pakan biasa dari tepung PARS. Diet aterogenik adalah formulasi pakan khusus yang ditujukan untuk membuat tikus mencapai keadaan hiperlipidemia dan dapat menimbulkan atau meningkatkan sel busa (*foam cell*) pada pembuluh darahnya. Formula ini dihasilkan dari penelitian terdahulu, yang terdiri dari: tepung PARS, Asam kolat, terigu, kolesterol dan minyak babi. Pakan tikus diberikan secara *ad libitum* pada setiap kotak kandang individu tikus sebanyak ± 40 gr/hari yang diganti dan ditimbang sisannya setiap hari. Ekstrak teh hijau dan teh merah diberikan pada tikus dengan cara disonde, dengan maksud agar semua hewan coba dalam kelompok perlakuan mendapatkan dosis yang sesuai dengan kelompoknya.

Hewan coba *Rattus norvegicus* strain *Wistar* dibagi secara random ke dalam 7 (tujuh) kelompok yaitu 1 kelompok pretest, 4 kelompok perlakuan yaitu diet aterogenik+teh merah 150 mg (A+TM150), diet aterogenik + teh merah 300 mg (A+TM300), diet aterogenik + teh hijau 150 mg (A+TH150), diet aterogenik+ teh hijau 300 mg (A+TH300), dan 2 kelompok kontrol yaitu kelompok diet normal (DN) dan kelompok diet aterogenik (DA). Masing-masing kelompok 4 (empat) ekor tikus. Lama perlakuan inti adalah selama 8 minggu. Parameter yang diukur adalah jumlah foam cell, jumlah ADMA, jumlah F2-isoprostan, dan aktivitas SOD pada jaringan aorta dan kadar ADMA, F2-isoprostan dan SOD pada serum. Data dianalisa secara multivariat dengan program statistik terkomputerisasi.

Hasil : Jumlah Foam cell, F2 Isoprostan, ADMA dan aktivitas SOD jaringan aorta serta kadar F2 Isoprostan, ADMA dan aktivitas SOD serum pada DA berbeda signifikan ($p<0,05$) dibandingkan DN. Secara univariat, jumlah Foam cell, F2 Isoprostan dan ADMA kelompok DA mengalami peningkatan baik pada jaringan maupun pada serum, sedangkan aktivitas SOD baik pada jaringan maupun serum mengalami penurunan tapi tidak signifikan. Pada semua kelompok perlakuan (A+TM150, A+TM300, A+TH150, A+TH300) ke-empat parameter tersebut menunjukkan perbedaan signifikan ($p<0,05$) dibandingkan DA. Penurunan ADMA jaringan terbesar adalah pada kelompok A+TM300. Penurunan F2 Isoprostan jaringan terbesar pada kelompok A+TM300. Pada Foam cell penurunan terbesar terjadi pada kelompok A+TH150. Pada F2 Isoprostan serum penurunan terbesar terjadi pada A+TM300. Penurunan ADMA serum yang terbesar adalah pada kelompok A+TM300. Peningkatan aktivitas SOD jaringan tertinggi pada A+TM150.. Peningkatan aktivitas SOD serum tertertinggi pada kelompok A+TM300. Hasil anilisis diskriminan menunjukkan bahwa variabel diskriminator aterogenesis pada jaringan adalah Foam cell, SOD dan F2-isoprostan, sedangkan variabel diskriminator aterogenesis pada serum adalah SOD.

Kesimpulan : Diet aterogenik yang diberikan selama 8 minggu berhasil menimbulkan suatu keadaan stres oksidatif dan aterogenesis pada tikus. Pemberian ekstrak teh hijau dan teh merah dapat memperbaiki keadaan stres oksidatif dan menghambat aterogenesis pada tikus dengan diet aterogenik. Mekanisme efek preventif ekstrak teh merah dan teh hijau terhadap aterosklerosis adalah dengan memperbaiki keadaan stress oksidatif dengan meningkatkan aktivitas SOD.

SUMMARY

ANTIOXIDANT POTENTIAL OF THE EXTRACT OF GREEN TEA (*Camellia sinensis*) AND RED TEA (*Hibiscus sabdariffa*) IN ATHEROGENESIS PROCESS IN RATS WITH ATHEROGENIC DIET

Tea is a drink that is universally consumed in many countries and various levels of community. In worldwide level, tea is the second most-commonly consumed liquid after water. Several epidemiological studies indicated that polyphenol consumption of green tea as well as other herbal polyphenol may reduce the incidence rate of degenerative diseases, particularly the risk of atherosclerosis. Therefore, the potential and the antioxidant mechanism of the extract of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) on the prevention of endothelial dysfunction should be studied further. The objective of this study was to disclose the preventive effect of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) extract to oxidative stress and atherogenesis.

By observing the potentials of red and green the as functional food, this study investigated further the potential and the mechanism of antioxidant preventive effect of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) extract to oxidative stress and atherogenesis. The outcome of this investigation may lead to the reduction of atherosclerotic risks, and finally, the reduction of the risk of cardiovascular disease. In addition, both green and red tea have soft image for healthy individuals as well as for those who have metabolic syndrome and the risk of coronary heart disease, since these commodities are basically not medicine, but regarded more as natural daily or regular drink with a wide spectrum of consumers, minimum side effects and allergic reactions, making those commodities easy for use for the therapy of to reduce the risk of degenerative diseases.

The objective of this study was to disclose the preventive effect of the administration of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) extract to oxidative stress and atherogenesis in rats given with atherogenic diet.

This was a laboratory experimental study, in which either samples (experimental animals) or treatment were controlled, measurable, and the effect of the treatment was more reliable. This study used randomized separate pretest-post-test control group design, since each time the analysis units, the blood and aortic tissue, were taken, the experimental animals should he sacrificed, not allowing the use of the same animals to obtain pretest and post-test data. The division of the experimental animals into seven groups, i.e., 1 pretest group, 4 treatment groups, and 2 control groups was undertaken randomly, so that all animals had the same opportunity to be allocated in all groups, in order to improve the internal validity.

The experimental animals used in this study were male Wistar strain *Rattus norvegicus*, aged ± 2 months. These animals are mammals whose physiological function of their body organs is similar to those of human beings. Additionally, its provision is cost-saving and their maintenance is uncomplicated.

There were two types of feed given to those rats during the study, i.e. normal/standard diet and atherogenic diet. Standard or normal diet are feed from PARS flour. Atherogenic diet is special high-lipid and cholesterol feed formulation modified to

render the rats to become hyperlipidemic, obese and to induce or enhance foam cells in the tissue of their blood vessel. The formula was produced in previous studies, comprising of PARS flour, colic acid, wheat, cholesterol, and swine oil. Rats feed was given ad libitum in each box of individual rats as much as ± 40 gr/day, which was replaced each day and the remnants were scaled. The extract of green and red tea was given to the rats with sonde, with the purpose that all experimental animals in treatment groups receive doses according to their own group.

The experimental animals of Wistar strain *Rattus norvegicus* were randomly divided into seven groups, 1 pretest group, 4 treatment groups, i.e., atherogenic and red tea of 150 mg (A+TM150), atherogenic and red tea of 300 mg (A+TM300), atherogenic and green tea 150 mg (A+TH150), and atherogenic and green tea 300 mg (A+TH300), and 2 control groups, normal diet (ND) and atherogenic diet (AD). Each group comprised four rats. The length of the treatment was 8 weeks. Parameters measured was foam cells count, ADMA level, F2-isoprostan, and SOD levels in aortic tissue, and ADMA, F2-isoprostan and SOD levels in the serum. Data were analyzed multivariately using computerized statistical program and discriminant analysis was undertaken to determine the predominant discriminator variable in atherogenesis process.

The result revealed that foam cell count in aortic tissue of AD group was significantly higher ($p < 0.05$) compared to that in ND group, and so were the F2 isoprostan and ADMA levels both in aortic tissue and serum. SOD activity either in aortic tissue or serum in AD group was lower but not significant than that in ND. In all treatment groups (A+TM150, A+TM300, A+TH150, A+TH300), the four parameters showed significant difference ($p < 0.05$) as compared to AD. The highest reduction of foam cells was found in A+TH150 group. The highest reduction of ADMA in tissue and serum was found in A+TM300 group. The highest reduction of F2 isoprostan in endothelial tissue and serum was found in A+TM300 group. The highest increase of SOD in aortic tissue and serum was found in A+TH300. The result of discriminant analysis revealed that the discriminator variable of atherogenesis in the tissue was foam cell, SOD, and F2-isoprostan, while the discriminator variable of atherogenesis in the serum was SOD.

In conclusion, atherogenic diet given for 8 weeks successfully induced an oxidative stress and atherogenesis in rats. The administration of green tea and red tea was able to improve oxidative stress and reduce the progress of atherogenesis in rats with atherogenic diet. The preventive mechanism of red and green tea extract against atherogenesis is by relieving oxidative stress through the increase of SOD activity.

ABSTRACT

ANTIOXIDANT POTENTIAL OF THE EXTRACT OF GREEN TEA (*Camellia sinensis*) AND RED TEA (*Hibiscus sabdariffa*) IN ATHEROGENESIS PROCESS IN RATS WITH ATHEROGENIC DIET

Tea is a drink that is consumed by various levels of community in many countries. Tea is the second most-commonly consumed liquid after water. Several epidemiological studies indicated that polyphenol consumption of green tea as well as other herbal polyphenol may reduce the incidence rate of degenerative diseases, particularly the risk of atherosclerosis. Therefore, the potential and the antioxidant mechanism of the extract of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) on the prevention of

endothelial dysfunction should be studied further. The potential antioxidant effect of green tea (*Camellia sinensis*) is catechin and red tea (*Hibiscus sabdariffa*) is anthocyanin.

The objective of this study was to disclose the preventive effect of the administration of green tea (*Camellia sinensis*) and red tea (*Hibiscus sabdariffa*) extract to oxidative stress and atherogenesis in rats given with atherogenic diet.

This was a laboratory experimental study using separate sample pretest-post-test control group design. The experimental animals of Wistar strain *Rattus norvegicus* were randomly divided into seven groups, 1 pretest group, 4 treatment groups, i.e., atherogenic and red tea of 150 mg (A+TM150), atherogenic and red tea of 300 mg (A+TM300), atherogenic and green tea 150 mg (A+TH150), and atherogenic and green tea 300 mg (A+TH300), and 2 control groups, normal diet (ND) and atherogenic diet (AD). Each group consist of four rats. The length of the treatment was 8 weeks. Parameters measured was foam cells count, ADMA level, F2-isoprostan, and SOD levels in aortic tissue, and ADMA, F2-isoprostan and SOD levels in the serum. Data were analyzed multivariately using computerized statistical program.

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Keywords: atherogenic diet, red tea (*Hibiscus sabdariffa*), green tea (*Camellia sinensis*), foam cell, ADMA, F2-isoprostan, SOD