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CINNAMOMUM BURMANNII PROTECTED HEPATOCYTES AND CORRECTED SERUM UREA AND URIC ACID LEVEL IN DIABETIC RATS.

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Abstract

Background. Hyperglycemia was reported to harm liver and induce metabolic inbalance. The tree bark of *Cinnamomum burmannii* (*C. burmanii*) was potential as anti-hiperglycemic agent. The bark was rich with secondary metabolites that may lead to possess other advantages for diabetes.

Objectives. To study the effect of aqueos extract of *C.burmannii* (AEC) on liver and metabolic condition by measuring necrotic hepatocytes, serum urea and uric acid levels in alloxan-induced diabetic rats (AIDRs).

Methods. Three groups of fasted rats (free allowed to water) were intraperitoneally injected with 150 mg/Kg BW in 1 ml normal saline 6 hours prior to intraperitoneal injection of 2 ml 10% glucose to induce AIDRs, the animals were allowed to 5% glucose solution for 24 hours afterward. The AIDRs were treated with 0.5 ml, 1ml, and 2 ml of AEC respectively for 2 weeks. Hematoxyline-Eosine-stained liver slices were observed for necrotic hepatocytes by light microscope. Serum urea and uric acid levels were measured by Glutamate dehydrogenase and Uricase-PAP methods respectively. Data were analyzed by ANOVA followed by LSD test, p values of < 0.05 were considered to be significant.

Results.

The given doses of AEC lowered urea and uric acid serum levels of AIDRs ($p = 0.000$) which approximated to the non diabetic rats's serum levels ($p > 0.05$). Except the smallest dose, the remaining doses of AEC reduced necrotic hepatocytes number in AIDRs ($p = 0.000$).

Conclusion.

Aqueos extract of *C.burmannii* protected hepatocytes and corrected serum urea and uric acid levels of alloxan-induced diabetic rats.

Keywords: *Cinnamomum burmanii*; diabetes; necrotic hepatocytes; serum urea: serum uric acid.

INTRODUCTION

Diabetes is a disorder characterized by chronic hyperglycaemia, and has commonly been subclassified into type 1 diabetes (T1DM) (with insulin-secreting β cells destruction by autoimmune mechanism) and type 2 diabetes (T2DM) (with insulin resistance and metabolic syndrome)[1]. Hyperglycemia has been reported to involve in activation of polyol pathway, AGE generation, ROS production, and RAGE activation[2,3], which collectively will lead to diabetic complications. ROS production associated with hyperglycemia have been shown to have significant role in the pathogenesis of liver function impairment and the development of NASH[4,5]. Liver biopsy on 136 patients at their first diagnoses of T2DM showed NASH was found in more than 90% of them and it was suggested that NASH may be early complication of T2DM on liver due to its pathogenesis correlating with insulin resistance.[6]

Active constituents of medicinal plants are being considered for potent antidiabetic effects.[7] Such plants contain substantial amounts of cardiac glicoside, phenolics, alkaloids, steroids, flavonoids, saponins, and tannins which are being suggested as potent agents against diabetes.[8] WHO recommended the evaluation of plants traditionally used to control diabetes because they are effective, non-toxic, with less or no side effects, and promoted them as candidates for oral therapy.[9] *Cinnamomum* was reported as a plant with many health benefits as it have properties such as anti-inflammatory, antimicrobial, antioxidant, antitumor, cholesterol-lowering and also have cardivascular, immunomudulatory , insulin mimetic , hypoglycemic effects.[10] There are 4 main species of *cinnamomum* namely: *C. zeylanicum, C. cassia, C. burmannii,* and *C. loureirii*. The inner bark of the plant contain aromatic essential oils with characteristic flavor and aroma. The higher the level of the oils , the stronger the flavor. *Cinnamomum* is rich in polyphenol which are natural antioxidant.[11] Some phenolic compunds in the essential oil of the four *cinnamomum* species have been mentioned above were cinnamaldehyde, coumarine, proanthocyanine, catechine, epicatechine, procyanidins B1, proanthocyanidins as major compounds. Among the four *cinnamomum* species, *C. burmannii* (grows in Indonesia) contains the highest amount of cinnamaldehyde and has a smoother taste with less bite than *C. cassia* and *C. loureirii*.[11] The combination of aqueous extract of *P. crocatum* (local name, *Sirih merah*) and of *C. burmannii* was traditionally used as functional beverage. A such combination was reported to have no harmful signs on liver, kidney, and blood of rats.[12] The suplementation of *C. burmannii* in cacao drink lead to return the antioxidant level of the drink.[13] *Cinnamomum* has been granted generally recognized as safe (GRAS) status as a food additive by the U.S. food and drug administration (FDA).[14] Many previous studies reported the active subtances of *cinnamomum* have anti-hyperglycemic action by mechanism of enhanced insulin secretion, inhibition of alanine absorption in the rat intestine (alanine plays important role in gluconeogenesis), insuline-like activity, enhanced insulin secretion, amelioration of oxidative stress in the pancreas.[15] The present study reported other benefit effects of *C. burmanii* inspite of its ability to correct blood glucose. This is an initial study of aqueous extract of *C. burmannii* (AEC) potency in affecting serum urea and

uric acid level, and hepatocytes conditions in aloxan induced-diabetic rats (AIDRs).

SUBJECTS AND METHODS

Plant collection and Extraction

C.burmannii powder was obtained from Materia Medica-Batu, Malang. The powder was dried, 10 g of it was mixed with 100 ml of water prior to save the solution in waterbath at 60°C for 2 hours. The aqueous extract was filtered to obtaine filtrat. Finally add the filtrat with aquadest until the volume up to 1000 ml. The amount of *C. burmannii* powder and extraction technique were adopted from Perdana et al, 2017. This study applaid 3 kinds of AEC doses namely; 0.5 ml, 1 ml, and 2 ml of which must have originated from aqueous extracts of 5 mg, 10 mg, and 20 mg of *C. burmannii* powder respectively.

Experimental Animals

Twenty five of male Wistar rats breeding at Biomedicine Laboratory of Brawijaya University. The ages were between 2 and 3 month, weighing about 200-300 g. The rats were randomly divided into 5 groups and housed in environment with a normal ambient temperature and humidity. Lighting period was 12 h daily. The research protocols were approved by Animal Ethical Commitee of Brawijaya University.

Induction of Experimental Diabetes and *C. burmannii* Administration.

18 hours-fasted rats (free access to water) were intraperitoneally injected with 150 mg/ Kg BW of aloxan in 1 ml of normal saline, 6 hours afterward the rats were intraperitoneally injected with 10 % glucose solution. Then the rats were free allowed to 5 % glucose solution for 24 hours. The tail blood glucose was detected by glucometer in 72 hours later. Hyperglycemia was exist when blood glucose > 200 mg/dl.

Three groups of AIDRs begin to recieve oral 0.5 ml, 1 ml, 2 ml of AEC respectively at day 4 after the intraperitoneally injection of 10% glucose solution. They recieve oral extracts every day for 14 days. The two other groups were healthy rats and AIDRs recieving no extract of *C. burmannii.* All groups of rats were sacrificed 18 days after alloxan injection.

Blood and Liver Collection

Rats were anesthesized by ether. Intracardiac blood was aspirated and put in EDTA containing centrifuge tubes then centrifuge at 3000 rpm for 10 minutes. Liver was gently cleaned by neutral buffer solution. The organs were put in 10% formaline for fixation. Blood and liver were saved in refrigerator at -4°C.

Measurement of Urea and Uric Acid Serum.

Urea serum: Urea serum was measured based on Coupled- enzyme calorimetric methods adopted from Sampson et al, 1980.[16] Briefly, preparing the enzymic reagent contains, per liter: Buffer pH 7,8 120 mmol/Ll, ADP 0.7 mmol/L, urease ≥ 7 kU/L, glutamate dehydrogenase 0.4 kU/L. The substrat reagent contains: 2-Oxoglutarat 5 mmol/L and NADH 0.25 mmol/L. The enzymatic and substrat reagents were stored at − 4°C and 4°C respectively for no longer than 15 days. 40 ml of enzymatic reagent and 10 ml of substrate reagent were mixed prior to be added into 10 µl serum or plasma then mixing the contents by gentle inversion. The samples were put in waterbath at 30°C for 30 minutes before reading with sphectrophotometer at λ 334 nm (the

cuvetes temperature were at 30°C). Absorbance reading were also done for standarts and blanko solution. The absorbance value was the difference absorbance value between sample and blanko solution. The values were converted into serum urea nitrogen concentrations by means of standart solution curve.

Uric acid serum: Uric acid serum was measured by Uricase-PAP method. Reagen composition: phosphate buffer solution 50 mmol/L, 4-Aminophenazone (PAP) 0.3 mmol/L, 3,5-dichloro-2-Hydroxybenzenesulfonic acid (DCHBS) 4 mmol/L, Uricase > 200 U/L, and peroxidase > 1000 U/L. Briefly; 20 µl of serum was mixed with the reagent then was incubated at 20-25°C for 10 minutes. Absorbance reading was at λ 546 nm. Standart of urea concentration- absorbance curve was used to find the urea serum concentrations.

Hepatic Tissue Slices Staining and Necrotic Hepatocytes measurement.

Liver was blocked with paraffin and cut the liver paraffine block by rotary microtome to obtaine 4 µm thick-slice. The slices were stained by Hematoxyline-Eosine. Necrotic hepatocytes of zona 1, 2, and 3 of lobulus were detected in 5 difference field of views by binocular light microscope (magnificient 1000X). Necrotic hepatocytes were characterized by membrane lysis, protein denaturation, eosinophilic cytoplasma, homogenous clear, and nuclear changes such as; karyolysis, picnosis, and karyorrhexis.

Data analysis

Data were statistical analyzed by ANOVA and followed by LSD test. Mean differences < 0.05 was considered to be significant.

RESULTS

Table 1. Mean±SD of necrotic hepatocytes numbers in zona 1, 2, dan 3 of liver lobulus of healhty rats, AIDRs, and AIDRs recieving AEC.

Groups of Rats	n	Mean±SD of necrotic hepatocytes numbers in respect with the lobulus zone		
		Zone 1	Zone 2	Zone 3
Healthy	5	0.520 ± 0.6419[a]	0.720 ± 0.6723*	0.440 ± 0.2608Ø
AIDRs	5	23.720 ± 3.5174[b]	24.360 ± 4.3460¶	25.360 ± 9.0768¤
AIDRs + 0.5 ml AEC	5	21.520 ± 3.4774[b]	22,520 ± 3.2668¶	23.560 ± 5.9387¤
AIDRs + 1 ml AEC	5	4.600 ± 3.3466[c]	3.600 ± 2.2091*	2.520 ± 3.4543Ø
AIDRs + 2 ml AEC	5	4.320 ± 2.3178[a]	3.560 ± 1.8515*	2.880 ± 1.9697Ø

Different notation in every mean values of each zone column indicates statistically significant difference ($p<0.05$). AEC, Aqueous extract of *C. burmannii*; AIDRs, Aloxan induced-diabetic rats.

Table 2. Mean±SD of serum level of urea, uric acid, and glucose of healthy rats, AIDRs and AIDRs recieving AEC.

Groups of Rats	n	Serum urea level (mg/dl)	Serum uric acid level (mg/dl)	Serum Glucose level (mg/dl)
Healthy	5	47.420 ± 7.9698*	1.540 ± 0.493*	114.80 ± 8.167*
AIDRs	5	156.680 ± 19.7003¶	2.460 ± 0.5177¶	431.40 ± 28.211¶
AIDRs + 0.5 ml AEC	5	57. 480 ± 5.2447*	1.340 ± 0.2702*	467.00 ± 66.765¶
AIDRs + 1 ml AEC	5	64.640 ± 18.0166*	1.380 ± 0.2490*	454.00 ± 62.940¶
AIDRs + 2 ml AEC	5	63.480 ± 24.2400*	1.340 ± 0.2793*	276.20 ± 197.994¥

*,¶,¥: Different notation presented statistically significant difference ($p<0.05$).

LSD test between AIDRs recieving AEC is unsignificantly different ($p>0,05$). AEC, Aqueous extract of *C. burmannii*; AIDRs, Aloxan induced diabetic rats.

DISCUSSION

Aqueous extract of C.burmannii reduced necrotic hepatocytes and serum glucose level of AIDRs.

The present study showed intraperitoneally injection of single dose alloxan at 150 mg/ KgBW significanly developed hyperglycemia and necrotic hepatocytes (table 1 and 2). A study used

intraperitoneal alloxan at dose of 120 mg/Kg BW to induce diabetes and the accompanying liver injury to study the effect of ethyl acetate fraction of *Sida cordata* on liver disorder.[17] Using alloxan at dose 42 mg/Kg BW administered into tail vein another research showed that alloxan-induced diabetes causes changes in rat livers that resemble the patogenesis of chronic fatty liver disease in humans.[18] Our previous study on alloxan (150 mg/Kg BW) induced diabetes of rats showed that iNOS serum level of AIDRs increased (p=0.007).[19] Inductive nitric oxide synthase (iNOS) catalized the reaction producing NO radicals playing a role in many diseases (including diabetes mellitus) in which the pathogenesis involve oxidative stress.[20] Experimental diabetes induced by intraperitoneal single dose of 150 mg/Kg BW alloxan were used to study histological changes of hepatocytes due to hyperglycemia.[21,22] As oxidative stress plays the crucial role in liver cells injuries caused by hyperglycemia, antioxidants are understandably considered to be a good therapeutic strategy for the treatment of liver disorders.[5] The present study showed oral AEC at dose of 2 ml has the best performance in reducing necrotic hepatocytes of AIDRs. The 2 ml dose of AEC reduced blood glucose but did not approximate the glucose level of the healthy rats (Table 2). Aqueous extract of *C. burmannii* bark has antioxidant activity higher than the ethanolic extract, the phytochemical analysis of AEC indicated that polyphenols (including flavonoid and tannin) and phenolic volatile oil compounds as the major antioxidant.[23]

Aqueous extract of *C.burmannii* lowered serum uric acid and urea level. Prospective cohort studies provided strong evidence that high level of serum uric acid is a risk factor for developing T2DM in middle aged and older people. Therefore, controlling hyperuricemia might be a future strategy for the T2DM prevention.[24]

A study of hyperuricemia prevalence of 641 T2DM patients (34-91 years) in Lagos, Nigeria provided it was 25%.[25] Hyperuricemia in T2DM is a risk factor for developing of cardiovascular complication,[26] retinophaty and albuminuria,[27], peripheral neurophaty.[28], and nephropathy.[29] Our study provided that intraperitoneally administration of single dose of 150 mg/KgBW alloxan significantly increased serum uric acid level of rats. The given doses of AEC reduced serum uric acid levels significantly compared with AIDRs recieving no AEC (Table 2). It was strongly supposed that high level of uric acid in alloxan- induced diabetic rats were caused by renal impairment.[30] Intraperitoneal administration of alloxan at dose 120 mg induced a deposit of eosinophilic materials in the intermediate substantial of medulla in the kidney of diabetic rats.[31] Uric acid is the end product of purin (guanosine, inosine, and adenosine) metabolism. A series of reaction were needed to converts purin into xantine. The latter will be oxidized into uric acid in a reaction catalized by xanthine oxidase (XOD).[32] In human, genes and high ingestion of purin, fructose, alcohol were factors increasing uric acid serum.[33,34] We did not measure AIDRs body weight, but Idakwoji,(2015) reported that intraperitoneal injection of single dose alloxan at 150 mg/kgBW lowered wistar rats body weight.[35] Respecting with this finding, it was stongly proposed that the reduction of the rat's body weight is accompanied with increased glycogenolysis, lipolysis, gluconeogenesis and these biochemical activities result in

loss of body tissue protein and fat.[36] A cross sectional study in croatians found the prevalence of hyperuricemia in adult is 10,7% and seems to be associated with prediabetic condition.[37] A Literature study reviewed the evidence that hyperuricemia may have a key role in the pathogenesis of insulin resistance by blocking endothelial nitric oxide supply. Thus it was speculated that hyperuricemia and insulin resistance share bidirectional causal effects.[38] The final product of protein catabolism is urea will be secreted through kidney.[39] Previous study reported that urea serum level of diabetic patient and alloxan induced-diabetes rats were increased due to kidney impairment.[40,41] Our study showed the three doses of AEC able to significantly reduced serum urea levels in AIDRs, as a note the serum urea levels after AEC administration were unsignificantly different compared with those of healthy rats (Tabel 2).

CONCLUSION

Aqueous extract of *C. burmannii* might has potency to protect hepatocyte necrosis, corrects serum urea and uric acid level in alloxan induced-diabetic rats due to diabetes mellitus in which oxidative stress may play role in the patogenesis.

SUGGESTIONS

We proposed to explore the optimum dose of aqueous extract of *C. burmannii* for reducing blood glucose on the Alloxan – induced diabetic rats.

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