Study of Hypoglicemic Effect and Acute Toxicity of Ethyl Acetat Fraction Tofu Residue

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Abstract

The study aimed to investigate oral glucose tolerance test (OGTT) and acute toxicity of Ethyl Acetat Fraction Tofu Residue (EAFTR) on DDY mice. Methods: EAFTR (200 mg/kg b.w) was prepared and administered orally to experimental animals used. The hypoglycemic effects of EAFTR on normal mice and orally glucose (3 g/kg bw) induced hyperglycemic mice were compared with aquadest, glibenclamide and dry tofu residue (200 mg/kg b.w). EAFTR was administered and blood glucose level was obtained by pricking the tail vein using glucometer at time -30, 0, 30, 60, 120 minutes. Hypoglicemic effect evaluated by Area Under Curve (AUC) measurement. The EAFTR was tested for acute toxicity in mice at a dose of 50, 500 and 2,000 mg/kg b.w. p.o once daily for 14 days. Results: The AUC of EAFTR treated groups were not different with control, Metformin treated and dry tofu residue. All doses of the EAFTR did not exert any sign or symptom of toxicity and the dead mice was not found. Conclusion: The findings of the present study can be concluded that EAFTR showed a hypoglicemic effect and did not show toxic symptom until dose 2000 mg/kg b.w.

***Keywords*:** *Blood glucose, Hypoglicemic Effect, acute Toxicity, Tofu Residue, Diabetes*

Introduction

This should be brief and indicates aim of the study and the essential back ground information. Introduction should clearly state the hypothesis or purpose statement, how and why the purpose or

hypothesis was developed and why the author deems it important.

Tofu is an important source of protein, made from soybean cruds. Over the past 25 years soyfoods have been rigorously investigated for their role in chronic disease prevention and treatment, because they are uniquely-rich sources of isoflavones[1]. Processing of tofu will produce solid and liquid waste. Solid waste is known as tofu residue. Tofu residue has traditionally been used as a food for a human and animal (fertilizer), although its disposal has become a significant problem in recent years[2].

Diabetes prevalence has been steadily rising more rapidly everywhere in middle and low income countries[3], especially Type 2 Diabetes Mellitus (T2DM). It can induce many serious health problems that appears to reduce life expectancy and enormous health costs. Its prevention should therefore be a worldwide priority. Tofu residue contains nutrient component about 50% fiber, 25% protein, and 10% lipid[4] and other components include isoflavones such as daidzein. Isoflavone extraction of tofu pulp effectively use the solvent ethyl acetate.

Various studies have been conducted concerning the beneficial role of dietary soy isoflavones and their metabolites to reduce hiperglikemic and other associated complications[5]. Many studies reported the prophylactic effect of daidzein on the improvement of hyperglycemia, insulin resistance, dislipidemia, obesity, inflammation, and other complications associated with T2DM[6-12]. However hypoglycemic effect and acute toxicity of Ethyl Acetat Fraction Tofu Residue (EAFTR) not yet been reported. The purpose of this research, therefore, was to examine the hypoglycemic effect and acute toxicity of Ethyl Acetat Fraction Tofu Residue (EAFTR) on DDY mice.

Material and methods

Preparation Of Ethyl Acetat Fraction Tofu Residue (EAFTR)

Tofu Residue from the same production batch was used for all experiments and it was supplied by Sumedang Bumi Armasta Tofu Factory, Bogor, Indonesia. Briefly, fresh tofu residue are dried by vacuum dry. After driying, the prepared tofu residue were extracted with HCl and Ethanol (1:8) by refluxing at 70ºC for 2 hr. After filtering through Whatman No. 41 filter paper, the filtrat obtained then concentrated using rotary evaporator. After that, filtrat were partial fractinated with ethyl acetate (1: 1) using separation tunnel, filtered and concentrated with rotary evaporator. The fraction was stored at -18˚C until use. Thin layer chromatographic screening of EAFTR using precoated Silica gel G 60 F254 plates (Merk). Detection was carried out using visible light, short and long UV lights before and after spraying with specific reagents. Colors and Rf values of detectable spots were recorded.

Animals

Animals Male and female DDY mice weighting 20-30 g which is purchased from the Biofarmaka, IPB, Bogor. The mice were housed under standard environmental conditions (at 25 ± 2°C, 40-60 % humidity with 12-h light/12-h dark cycle). All animals were given a standard laboratory diet with access to water

ad libitum. The experimental protocol and the experiments performed on the rats were approved by the Institutional Animal Care and Use Ethic Committee, Pakuan University, Indonesia (License No. No.16/KEPHP-UNPAK/3-2019).

Oral glucose tolerance test (OGTT)

Distilled water, EAFTER (200 mg/kg), Dry tofu resiude (DTR) and Metformin (65 mg/kg) were administered to four groups of mice, respectively. Glucose (3 g/kg) was fed 30 min after pretreatment with distilled water, EAFTR, DTR and Metformin. Blood glucose levels were measured at 0, 60, 90, and 120 min after glucose load to access the effect of extract on blood glucose levels of the glucose loaded animals. The blood glucose was measured using blood glucose test strips and glucometer (Easy touch; Miaoli County,Taiwan).

Acute toxicity test

Acute toxicity test fifteen of the test animals per group were fasted overnight (12 h) and weighed. Test doses of EAFTR were calculated in relation to the body weight of every fasted animal and administered on a doses of extract 0, 1000 1500 and 2000 mg/kg respectively. The animals were regularly and individually observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days. Mortality and body weights were recorded.

Statistical analysis

Hypoglicemic effect evaluated by Area Under Curve (AUC) measurement. All data were expressed as mean + standard error of mean (SEM). Statistical analysis was carried out using One-Way ANOVA by Duncan test. The criterion for statistical significance was at a p-value less than 0.05.

Result and Discussion

Phytochemical analysis

Phytochemical analysis have shown that EAFTR has flavonoid content. The TLC result shows the stains that EAFTR have Rf of about 0.66 and RF daidzein standart about 0.68. The Result shown at Fig 1.

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test (OGTT) Oral Glucose Tolerance Test, the blood samples were analyzed for glucose content at 0, 60, 90 and 120 minutes, respectively. The AUC of EAFTR (200 mg/kgbb) treated groups not different with control, Metformin treated and dry tofu residue. The Result shown at Fig 2.

Acute toxicity

The limit dose of 2000 mg/kg did not cause mortality or any sign and symptom of acute toxicity in the fifteen mices dosed for a short period (24 h) and long period (14 days).

DISCUSSION

Soybean has become noteworthy in recent years. Many studies have been reported on the beneficial components of soybean such as protein, phytochemicals, and peptides. Bakhtiari has reported the beneficial effects of administration soy protein in elderly women with borderline parameters of Metabolic syndrom who suffered from a hyperlipidemic, insulin resistance status (TG/HDL-C > 3.5, HOMA-IR > 2.5 and fasting insulin > 12.5) and oxidative stress (MDA ≥ 5). Soy in their usual diet can reduce the need for medical treatment through the simultaneous improvement of multiple metabolic disorders. Its tofu residue, also contains these components, and we have shown here the beneficial effects of an tofu residue intake on the hypoglicemic effect and status of acute toxicity.

The oral glucose tolerance test (OGTT) is a widely used to evaluate apparent insulin release and insulin resistance in various clinical settings. For Oral Glucose Tolerance Test, the blood samples were analyzed for glucose content at 0, 60, 90 and 120 minutes, respectively. The single dosed study of EAFTR, doses at 200 mg/kg produced no significant hypoglycemic effect in normal mices. It's was compared with Metformin has been used for many years to treat diabetes and increase insulin sensitization.

Various studies have been conducted concerning the beneficial role of dietary soy isoflavones and their metabolites to reduce hiperglikemic and other associated complications[5]. Many studies reported the prophylactic effect of genistein and daidzein on the improvement of hyperglycemia, insulin resistance, dislipidemia, obesity, inflammation, and other complications associated with T2DM[6-12].

EAFTR have daidzein content, shown by TLC. The result show in Fig 1. Ni'mah reported that EAFTR contained 65.93 mg/100g genistein and 63.68 mg/100g daidzein[13]. Several studies have confirmed that daidzein promotes glucose uptake in adipocytes and muscle cells and suppresses the rise of serum glucose level 6,7, 8. Cheong et al. [10] reported the promising role of equol, a daidzein derivative, in serum glucose uptake via GLUT4 translocation, and AMPK activation. During in vivo studies, equol was found to regulate the expression levels of major genes involved in gluconeogenesis and glycogenesis pathways. Equol treatment significantly decreased the expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) and increased the glycogen synthase (GS) and enhanced basal glucose uptake in high glucose diet-fed mice.

In another study, Cheong et al[7] reported the beneficial effect of daidzein in improving glucose homeostasis in high glucose treated L6 myotube cells. Results showed that treatment with the optimum dose of daidzein caused a significant elevation in the ratio of glucose transporter 4 (GLUT4) to Na+/K+ ATPase in the plasma membrane (PM) fraction of L6 myotubes suggesting the potential role of daidzein in promoting glucose uptake via GLUT4 translocation from intracellular microvesicles to PM. Moreover, the authors also investigated the role of adenosine monophosphate activated protein kinase (AMPK), the key regulator of cellular energy homeostasis in mediating the beneficial action of daidzein on glucose uptake. It was found that daidzein supplementation led to a significant phosphorylation of AMPK in high glucose treated myotubes, which in turn promoted GLUT4 translocation and subsequent glucose uptake.

Using adipocyte cell culture model, Sakamoto et al.[8] reported that treatment with daidzein upregulated the adipogenic differentiation and gene expression of PPARγ and adiponectin and downregulated the MCP-1 gene expression and its secretion. Additionally, the authors reported that in high fat diet-fed mice daidzein administration also upregulated the gene expression of PPARγ and adiponectin and downregulated the MCP-1 and TNFα gene expression in fat tissue and thereby inhibited hypertrophy in fat cell size and improved insulin sensitivity.

Toxicity study is required not only to identify the further range of doses in animal studies but also to explain the probable clinical signs evoked by the test compounds under investigation[14]. The results obtained from the acute toxicity study showed that the EAFTR demonstrated high safety margin since the animals tolerated up to 2000 mg/kg body weight of the extract orally. However, further studies are needed to confirm long-term toxicities.

Conclusion

The findings of the present study can be concluded that the Ethyl acetat fraction of tofu residu (EAFTR) have hypoglicemic effect, doses at 200 mg/kg b.w produced no significant hypoglycemic effect in normal rats.

A study in acute toxicity EAFTR are practically non-toxic at a lower dose which one 2,000 mg/kg.

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Acknowledgement

The authors gratefully acknowledge the Institute of Research and Community Service (LPPM), Pakuan University and the Ministry of Research, Technology and Higher Education For partial financial support.

Tables & Figures

FIGURE

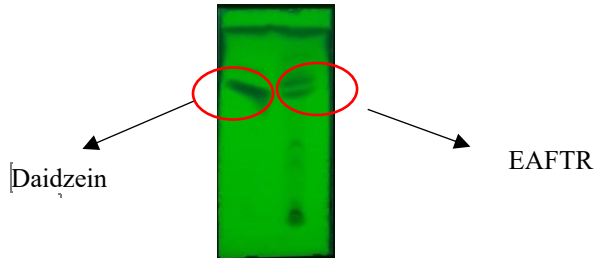

Figure 1. Thin Layer Chromatography Result

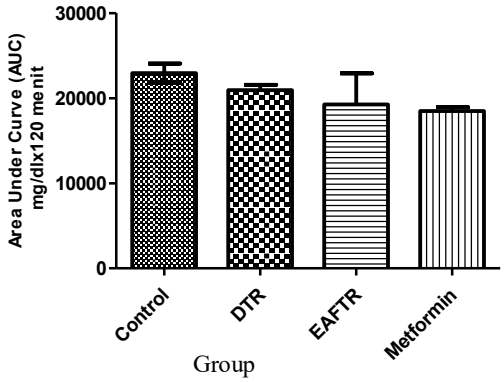

Figure 2. Effect of FEAAT on glucose metabolism in vivo. The area under the data curve (AUC) is calculated for TTGO. Mice were given glucose orally at 2 g kg / kg body weight. Blood glucose levels are measured in tail vein blood at 0, 30, 60, 90, and 120 minutes. The data set displayed is representative of three experiments. Data is represented as mean ± SD (n = 3 / group).