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Original Research

Hypoglycemic and hypolipidemic effects of unsaponifiable matter from okra seed in diabetic rats

Dongyeon Seo [1*], Naeun Kim [1*], Ahyeong Jeon [1*], Jihyun Kwon [1], In-hwan Baek [2], Eui-Cheol Shin [3], Junsoo Lee [4], and Younghwa Kim [1§]

[1]Department of Food Science and Biotechnology, Kyungsung University, Busan 48434, Korea
[2]College of Pharmacy, Kyungsung University, Busan 48434, Korea
[3]Department of GreenBio Science/Food Science and Technology, Gyeongsang National University, Jinju 52725, Korea
[4]Department of Food Science and Biotechnology, Chungbuk National University, Cheongju 28644, Korea

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§Corresponding Author:
Younghwa Kim
Department of Food Science and Biotechnology, Kyungsung University, 309 Suyeong-ro, Nam-gu, Busan 48434, Korea.
Tel. +82-51-663-4652
Fax. +82-51-633-4709
Email. younghwakim@ks.ac.kr

*Dongyeon Seo, Naeun Kim and Ahyeong Jeon contributed equally to this work.

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ORCID iDs
Dongyeon Seo
https://orcid.org/0000-0002-0243-6671
Naeun Kim
https://orcid.org/0000-0002-0217-9337
Ahyeong Jeon
https://orcid.org/0000-0003-3721-753X
Jihyun Kwon
https://orcid.org/0000-0003-0459-6676
In-hwan Baek
https://orcid.org/0000-0002-9538-0565
Eui-Cheol Shin
https://orcid.org/0000-0003-4243-4643

ABSTRACT

BACKGROUND/OBJECTIVES: Okra seed is a rich source of various nutritional and bioactive constituents, but its mechanism of action is still unclear. The aim of this study was to evaluated the effects on glucose uptake and serum lipid profiles of unsaponifiable matter (USM) from okra seed in adipocytes and diabetic animal models.
MATERIALS/METHODS: USM was prepared from okra seed powder by saponification. The contents of phytosterols and vitamin E in USM were measured. 3T3-L1 preadipocytes were cultured for 6 days with different concentrations of USM (0–200 μg/mL). The diabetic rats were administered with or without USM for 5 wk.
RESULTS: In the USM, the contents of phytosterols and vitamin E were 394.13 mg/g USM and 31.16 mg/g USM, respectively. USM showed no cytotoxicity and led to an approximately 1.4-fold increase in glucose uptake in 3T3-L1 adipocytes. The treatment of USM also increased the expressions of peroxisome proliferator-activated receptor-γ and glucose transporter-4 in a dose-dependent manner in adipocytes. The body weight change was not significantly different in all diabetic rats. However, blood glucose and the weights of liver and adipose tissues were significantly reduced compared to those in the control diabetic rats. Treatment with USM decreased the levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol compared to the control group. The USM group also showed significantly decreased atherogenic indices and cardiac risk factors.
CONCLUSION: These results suggest that USM from okra seed improves the hypoglycemic and hypolipidemic effects in diabetic rats, and provides valuable information for improving the functional properties of okra seed.

Keywords: Okra; seeds; phytosterols; tocopherols; hypoglycemia

INTRODUCTION

Obesity is a chronic metabolic disorder characterized by the accumulation and deposition of excess triglyceride (TG) in adipose tissues [1]. Obesity is closely related to lead the various diseases such as type 2 diabetes, cardiovascular disease, hypertension, hyperglycemia and hyperlipidemia [2]. Recently, several studies on adipocytes have been conducting, and adipocytes are emerging as a major therapeutic target for obesity-related metabolic diseases

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Junsoo Lee
https://orcid.org/0000-0001-5557-4229
Younghwa Kim
https://orcid.org/0000-0003-4186-887X

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Conflict of Interest
The authors declare no potential conflicts of interests.

Author Contributions
Conceptualization: Kim Y; Formal analysis: Seo D, Jeon A, Kim N; Funding acquisition: Kim Y; Investigation: Seo D, Jeon A, Kim N, Kwon J, Baek IH, Shin EC, Lee J; Visualization: Jeon A, Kim N; Writing - original draft: Seo D, Jeon A, Kim N; Writing - review & editing: Kim Y.

[3]. A number of transcription factors including peroxisome proliferator-activated receptor-γ (PPARγ), glucose transporter-4 (GLUT4), and leptin are well-known to be involved in the adipogenesis, glucose uptake, and glycolysis pathway [4]. PPARγ regulates the expression of genes associated with insulin signaling and glucose and lipid metabolism in mature adipocytes. GLUT4 is predominantly expressed in insulin-sensitive tissues such as muscle and adipocytes as a high-affinity glucose transporter [5].

Type 2 diabetes, which constitutes almost 90% of diabetic population, is caused by impaired insulin action, generally mentioned as insulin resistance or insensibility [6]. It is a multiple metabolic disorder characterized by hyperglycemia along with damaged metabolism of glucose, lipid and protein [7]. Furthermore, hyperglycemia can produce a considerable amount of the oxidative stress by various mechanisms, containing non-enzymatic protein glycation, glucose auto-oxidation [8]. Hyperlipidemia, which is regarded as a major risk factor for cardiovascular diseases, is characterized by higher total cholesterol (TC), TG, and low-density lipoprotein cholesterol (LDL-C) levels, as well as lower high-density lipoprotein cholesterol (HDL-C) level [8]. Recently, it was reported that natural bioactive food components with less pronounced side effects could be useful for the control of fat accumulation in liver, hyperlipidemia, and blood glucose concentrations [9,10].

Okra (*Abelmoschus esculentus* (L.) Moench) is an important vegetable crop widely grown in subtropical, tropical and warm temperate countries [11]. The immature fruits are consumed as vegetables, however, over-matured okra pods are generally not palatable and discarded resulting in substantial post-harvest waste. Okra seed, which comprises approximately 15% of the total weight of an okra fruit, is discarded because it is too hard to use as food resource. Interestingly, okra seed is a rich source of various nutritional and bioactive constituents such as essential amino acids, linoleic acid, phytosterols, vitamin E, and flavonoids, which possess beneficial effects on human health due to their antioxidant activity, anti-cancer, anti-coronary heart disease, and hypocholesterolemic properties [12]. The unsaponifiable matter (USM) is defined as substances that are dissolved in lipids that are insoluble in aqueous solution but soluble in organic solvent after saponification. The USM from vegetable oil contains significant amounts of tocopherols, sterols, other phenolic compounds and tocotrienols [13]. Previously, the USM showed a inhibition of photoaging and fat accumulation in the liver and epididymal tissue and decreasing serum lipid levels in high-fat diet rats [14,15]. In a previous study performed in our laboratory, the antioxidant activity and phytochemicals contents of okra seed oil were investigated [16]. Therefore, the present study was aimed to evaluate the phytochemical content of USM from matured okra seeds and the hypoglycemic and hypolipidemic effects were also investigated in 3T3-L1 adipocytes and animal models.

MATERIALS AND METHODS

Chemicals

The matured okra seed were collected from Buyeo, Korea and stored at 4°C until use. Dulbecco's modified Eagle's medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), bovine serum (BS), trypsin-EDTA and penicillin-streptomycin were purchased from Gibco (Thermo Fisher Scientific, Lafayette, CO, USA). Rosiglitazone was from Cayman (Ann Arbor, MI, USA). PPARγ, GLUT4, aP2, β-actin and anti-mouse antibodies were obtained from Santa Cruz (Dallas, TX, USA), 4 X Laemmli sample buffer, and ECL was purchased from

BioRad (Hercules, CA, USA). Cell lysis buffer was bought from iNtRON (Seoul, Korea) and protein marker was from Bioprince (Seoul, Korea).

Preparation of USM from okra seed

The okra seed was washed and dried, grinded to using a blender. Approximately 2 g of the okra seed powders were mixed with 10 mL of 6% pyrogallol in ethanol, and the mixture was flushed with nitrogen gas. After sonicating for 5 min, 8 mL of 60% potassium hydroxide in distilled water was added. The mixture was shaken at 100 rpm for an hour at 75°C in a shaking water bath. After cooling the mixture in an ice bath, 2% sodium chloride in distilled water was added and mixed. The resulting solution was extracted with 30 mL of hexane/ethyl acetate (85:15, v/v) three times. The ethyl acetate/hexane layer was collected, filtered, and evaporated with rotary evaporator (EYELA N-1000; Rikakikai Co., Tokyo, Japan). Then the residue was dissolved in dimethyl sulfoxide (DMSO) and stored at −20°C.

Determination of phytosterols and vitamin E contents in the okra seed USM

The phytosterol contents were determined according to the procedure described by Shin *et al.* [17]. To measure the contents of phytosterols, one gram of USM was mixed with 500 μL of 5 α-cholestane as an internal standard. The USM was mixed with N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and pyridine solution for an hour. The content of phytosterols, was measured by gas chromatography (7890A GC; Agilent, Santa Clara, CA, USA) using a SACTM-5 capillary column (30 m × 0.32 mm i.d.; Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector. The column was initially maintained at 280°C for 1 min, increased to 300°C at 2°C/min, and then held at 300°C for 20 min. The flame ionized detector and injector were set at 310°C and 320°C, respectively, with a split ratio of 50:1 under helium atmosphere at a flow rate of 1.0 mL/min.

The vitamin E contents were determined using to HPLC-FLD (2000 series; Jasco Corporation, Tokyo, Japan). About one gram of the USM was diluted in 50 mL hexane and diluted USM was filtered by using 0.45 μm PTFE filter (Whatman™; Dassel, Germany). Tocopherol and tocotrienol standards were obtained from Merck KGaA (Darmstadt, Germany). A LiChrosphere® Diol column (250 × 4 mm i.d., 5 μm; Merck KGaA) was used with a mobile phase of 1.2% isopropanol in n-hexane at a flow rate of 1.0 mL/min. The wavelengths were 290 nm for excitation and 320 nm for emission [18].

3T3-L1 adipocyte differentiation and cytotoxicity

3T3-L1 preadipocytes (ATCC®, CL-173; ATCC, Manassas, VA, USA) were grown in DMEM supplemented with 10% BS and 1% penicillin/streptomycin at 37°C in a 5% CO_2 incubator, according to previous study [19]. 3T3-L1 preadipocytes were maintained in DMEM containing 10% BS until confluent. At two days postconfluence (day 0), the cell differentiation was induced by a mixture of dexamethasone (1 μM), IBMX (0.5 mM), and insulin (1 μg/mL) in DMEM containing 10% FBS. On day 2, this medium was changed with DMEM containing 10% FBS and insulin only. On day 4, the medium was replaced with DMEM containing 10% FBS only. Cytotoxicity was measured using the MTT assay [20]. 3T3-L1 cells were grown in cell culture plates. After, cells were treated with different concentrations (25, 50, 100, and 200 μg/mL) of USM at 37°C for 24 h with 5% CO_2. Then, MTT solution (4 mg/mL) was added to each well, and the plates were further incubated at incubator. After the incubation for 2 h, MTT reagent was removed, and its violet formazan crystals were dissolved in DMSO. The absorbance was measured at 550 nm using an ELISA reader (Thermo Fisher Scientific).

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Glucose uptake

In 96-well plates, 3T3-L1 preadipocytes were induced to mature adipocytes and incubated with the medium to FBS-DMEM supplemented with 1 μg/mL insulin, 0.5 mM IBMX and 1 μM dexamethasone for 48 h. After differentiation, the adipocytes were incubated with the medium to FBS-DMEM supplemented with insulin (1 μg/mL) for 48 h. On day 6 of differentiation, the mature adipocytes were cultured with serum free/low glucose DMEM for 18 h. After removing the medium of starved cells, the cells were washed with PBS two times and cultured with USM (200 μg/mL) and rosiglitazone (1 μM) in Krebs-Ringer phosphate-HEPES buffer supplemented with 2% BS albumin. Dissolution of 2-deoxyglucose (2-DG) was induced by using the glucose uptake fluorometric assay kit (Biovision, Zurich, Switzerland). The uptake of 2-DG was measured using a fluorescence spectrophotometer (BioTek, Winooski, VT, USA) with excitation and emission wavelengths of 532 nm and 587 nm, respectively.

Western blot analysis

Western blot was performed to investigate the expression of PPARγ, aP2 and GLUT4 by the okra seed USM. 3T3-L1 cells were treated with different concentrations (0, 25, 50, 100, 200 μg/mL) of USM for 6 days. Using a cell lysis buffer, the cells were extracted to obtain total protein. Protein lysates were then analyzed by SDS-PAGE, transferred to nitrocellulose membrane, and then blocked using a 5% non-fat dry milk-tris-bufferd saline and Tween20 (TBST) overnight in 4°C. After washing with TBST, the membranes were incubated with primary antibodies against PPARγ, aP2 and GLUT4 for 1 h. After washing with TBST three times, the membranes were incubated with secondary antibodies at room temperature for 1 h, then developed with ECL detection reagents (GE Healthcare, Chicago, IL, USA). The quantitative analysis of the bands was performed by ImageJ (NIH, Rockville, MD, USA).

Real-time polymerase chain reaction (PCR)

3T3-L1 cells were treated with okra seed USM (25, 50, 100, and 200 μg/mL) for 6 days and the RNA was isolated using the TRIsure reagent (Bioline, Alexandria, Australia) according to the manufacturer's instructions. cDNAs were synthesised from 2 μg of mRNA using the cDNA kit (Thermo Fisher Scientific). To evaluate the expression of PPARγ (Mm00440940_m1) and GLUT4 (Mm00436610_g1) mRNA, a Taqman assay was performed using Step-one plus real time-PCR (Thermo Fisher Scientific). And β-actin (Mm00607939_s1) was used as housekeeping gene.

Animal experiments and sample administrations

Diabetic Goto-Kakizaki (GK) rat were obtained from Jung-Ang Lab Animal Inc. (Seoul, Korea). The animals were housed under constant conditions (21–25°C; 40–60% humidity; 12-h light/12-h dark cycle; 10 times/h air ventilation) and allowed free access to food (general chow, Jung-Ang Lab Animal Inc., Seoul, Korea) and water for 2 wk. All rats used in the experiments were randomly allocated into three groups (n = 8 in each group): untreated GK rats group (control group), GK rats treated with 10 mg USM/kg (USM group), and GK rats treated with 30 mg quercetin/kg (QUE group) as positive control. Treatment concentrations of USM and quercetin were determined according to previous study [21-23]. Samples were diluted in 1% carboxymethyl cellulose (CMC) and orally administered once a day using oral gavage, while the control rats were given 1% CMC water via oral gavage for 5 wk. The body weight and blood glucose were measured once a four days. Blood glucose was measured by using the blood sample were taken from the tail vein. Animal studies were performed in line with the principles outlined in the National Institutes of Health Guide for the Care and Use

of Laboratory Animals, and the study design was approved by the animal ethics committee of Kyungsung University (KSU research-19-011A).

Sample collection

After 5 wk of administration, all rats were fasted for 12 h and exposed to diethyl ether. Blood for plasma biochemical analysis was collected in Vacutainer SST tubes (BD Bioscience; Franklin Lakes, NJ, USA). The plasma was obtained by centrifuging the blood at 10,000 × g for 5 min at 4°C. The abdominal fat, epididymal fat and liver tissues were carefully excised, rinsed in cold physiological saline solution, and weighed.

Analysis of plasma biochemical parameters

TC, HDL-C, LDL-C, TG, alanine transaminase (ALT), blood urea nitrogen (BUN), creatinine, and glucose levels in serum were analyzed by using automated chemistry analyzer (Cobas c111, F. Hoffmann-La Roche Ltd., Basel, Switzerland). The atherogenic index (AI) and the cardiac risk factor (CRF) were calculated according to the following formulas [24].

$$AI = (TC - HDL\text{-}C)/HDL\text{-}C$$
$$CRF = TC/HDL\text{-}C$$

Statistical analysis

The results are expressed as the mean ± standard deviation and are representative of 3 or more independent experiments. Statistical analysis was performed using the one-way analysis of variance (ANOVA) followed by Tukey's comparison test using GraphPad Prism 4.0 software (GraphPad, San Diego, CA, USA).

RESULTS

Contents of phytosterols and vitamin E in USM from okra seed

The extraction yield of USM from okra seed was 0.48 ± 0.01%. In this study, phytosterols and viamin E, which are major components of USM, were analyzed (**Table 1**). In the results, total phytosterol content was 394.13 mg/g USM, and the predominant phytosterol was β-sitosterol

Table 1. Contents of phytosterols and vitamin E in unsaponifiable matter from okra seed

Components	Contents (mg/g USM)
Phytosterols	
Campesterol	50.41 ± 0.52
Stigmasterol	9.21 ± 0.89
β-Sitosterol	320.39 ± 6.57
δ5-Avenasterol	14.12 ± 1.68
Total	394.13 ± 5.26
Vitamin E	
α-Tocopherol	17.45 ± 0.85
β-Tocopherol	nd
γ-Tocopherol	13.97 ± 0.36
δ-Tocopherol	nd
α-Tocotrienol	nd
β-Tocotrienol	nd
γ-Tocotrienol	nd
δ-Tocotrienol	nd
Total	31.16 ± 1.21

The results are expressed as means ± SD.
USM, unsaponifiable matter; nd, not detected.

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(320.39 mg/g). In the USM, only two forms of tocopherol isomers, α- and γ-tocopherol, were observed; however, other forms of vitamin E, such as β-tocopherol, δ-tocopherol, and all tocotrienols, were not detected. The content of α-tocopherol was 17.45 mg/g, and it was higher than the value of γ-tocopherol. From these results, phytosterols and tocopherols were the main components, accounting for approximately 43% of the USM.

Effect of the okra seed USM on glucose uptake

The cytotoxicity of USM was assessed using the MTT assay in 3T3-L1 cells. USM had no cytotoxicity at concentrations up to 200 μg/mL in 3T3-L1 cells for 6 days (**Fig. 1A**). Then, we investigated whether USM modulates the activity of glucose uptake in 3T3-L1 adipocytes. In the results, USM from okra seed significantly increased glucose uptake activity compared to control cells (**Fig. 1B**). In 3T3-L1 adipocytes, 1.34 and 1.17-fold increases in 2-DG uptake resulted after treatment with 200 ug/mL USM and rosiglitazone, respectively, when compared to the untreated control adipocytes. As seen in **Fig. 2**, the protein expression of PPARγ and GLUT4 in adipocytes was markedly increased by USM treatment. It showed that USM could have an effect on the promotion of glucose uptake in adipocytes.

Effects of USM on the level of blood glucose and weights of liver, abdominal and epididymal adipose tissue

To investigate the effect of USM on hypoglycemic and hypolipidemic activities in diabetes rats, USM was administered daily for 5 wk. As shown in **Fig. 3A**, the supplementation of USM and quercetin did not show any significant changes in body weight. The blood glucose levels were measured once every two days for 5 wk (**Fig. 3B**). There were no statistically significant differences in blood glucose levels among the groups at the beginning of the study. However, GK rats administered with USM and QUE were significantly reduced the glucose levels at the end of the experiment compared to the control group. The liver, abdominal and epididymal fat weights of the animals were weighed at the end of the study and shown in **Fig. 4**. USM treatment decreased the weights of liver, abdominal fat, and epididymal fat compared to control group. There were no significant differences in the liver and abdominal fat weights of QUE group compared to control group, however the epididymal fat weight of QUE group was significantly decreased. In these results, the liver, abdominal and epididymal fat weight in USM-treated group revealed significant lower levels compared to control group.

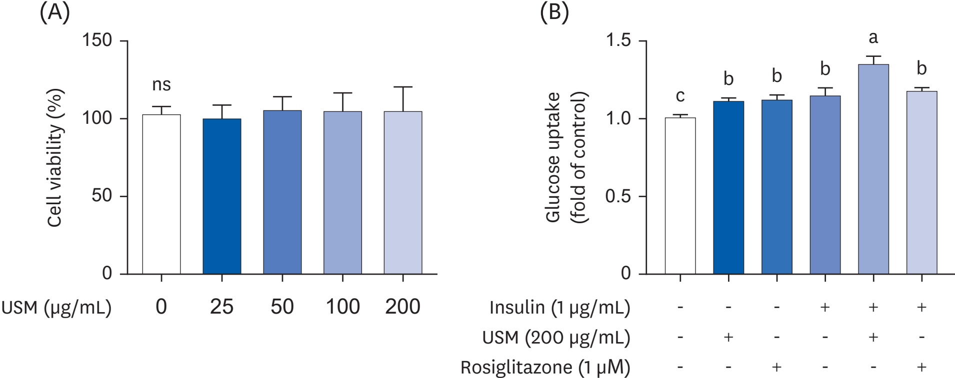

Fig. 1. Effects of USM from okra seed on cytotoxicity (A) and 2-deoxyglucose uptake (B) in 3T3-L1 adipocytes. The results are expressed as means ± SD. Different letters above bars indicate significant difference ($P < 0.05$). USM, unsaponifiable matter; ns, not significant.

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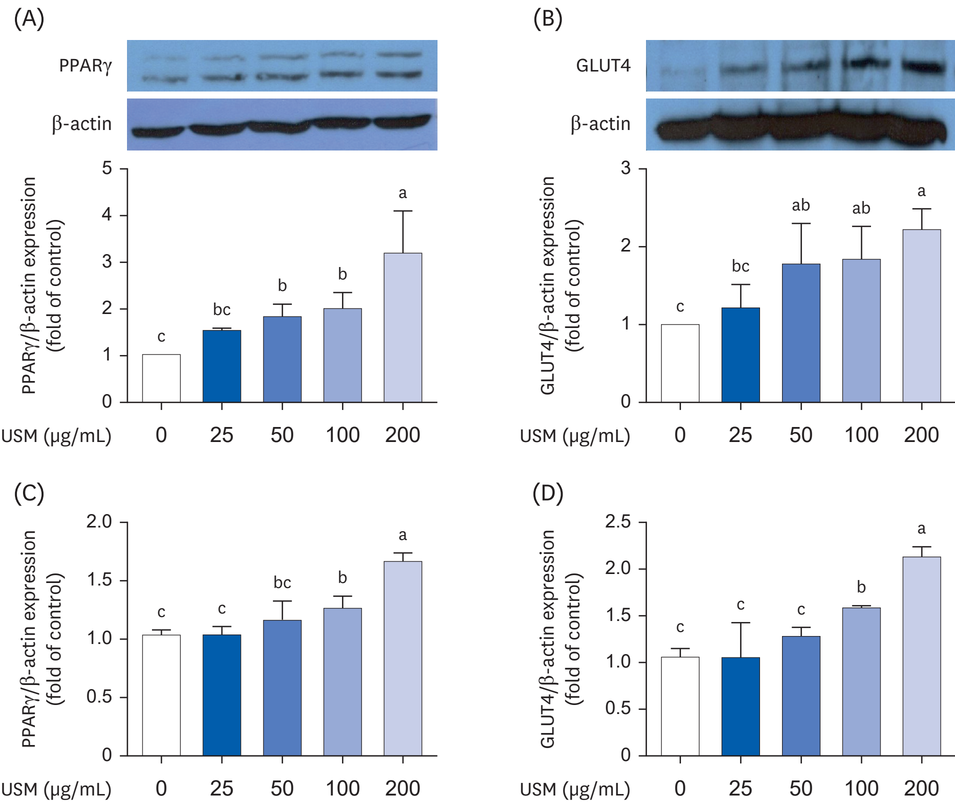

Fig. 2. Effects of USM from okra seed on PPARγ (A) and GLUT4 (B) protein expressions, and PPARγ (C) and GLUT4 (D) mRNA expression in 3T3-L1 adipocytes. The results are expressed as means ± SD. Mean values with different superscripts are significantly different ($P < 0.05$).
USM, unsaponifiable matter; PPARγ, peroxisome proliferator-activated receptor-γ; GLUT4, glucose transporter-4.

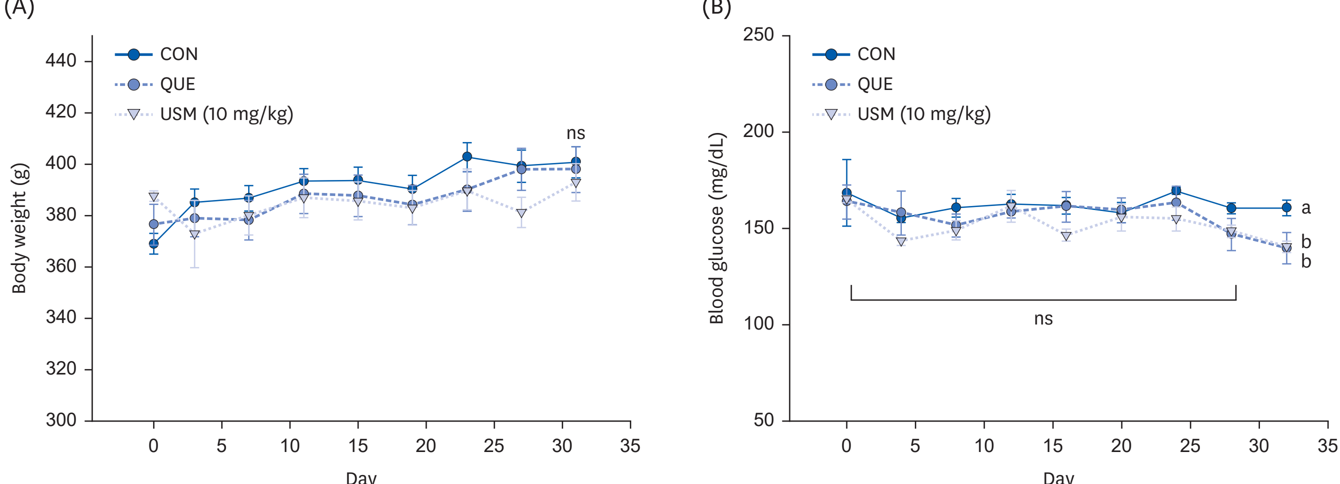

Fig. 3. Effect of unsaponifiable matter (10 mg/kg) from okra seed on body weight (A) and blood glucose levels (B). The results are expressed as means ± SD. Mean values with different superscripts are significantly different ($P < 0.05$). Quercetin was used as positive control sample (30 mg quercetin/kg).
CON, control; QUE, quercetin; USM, unsaponifiable matter; ns, not significant.

Effects of USM on biochemical parameters of blood plasma

Serum lipid profiles were examined and shown in **Fig. 5**. As a result, USM group showed significant differences from the control in the results of all parameter except for BUN and creatinine levels. The administration of USM significantly reduced lipid metabolic

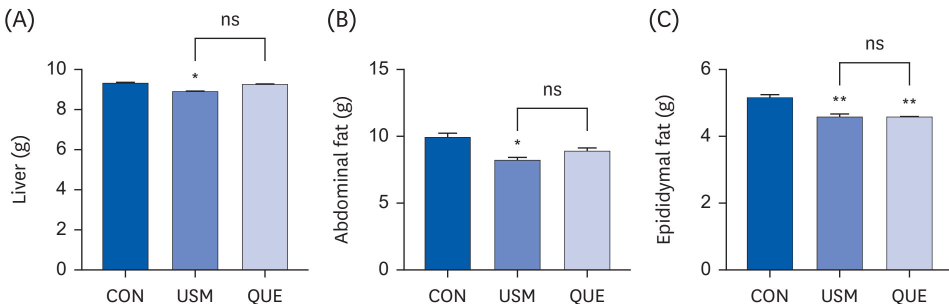

Fig. 4. Effect of unsaponifiable matter (10 mg/kg) from okra seed on liver (A), epididymal fat (B) and abdominal fat weight. The results are expressed as means ± SD. Quercetin was used as positive control sample (30 mg/kg).
CON, control; USM, unsaponifiable matter; QUE, quercetin; ns, not significant; ns, not significant.
Asterisk (*) indicates a significant difference versus the control group ($^{*}P < 0.05$, $^{**}P < 0.01$).

parameters such as TG, TC, and LDL-C compared to control group. The TG, TC, and LDL-C levels in the QUE group was decreased compared with control groups, however, there was no significant difference. USM treatment exhibited significantly reduced levels of glucose, ALT, and TG compared to QUE treatment. The treatment of USM induced beneficial effects in increasing the level of HDL-C compared to control group. Glucose level of the rat in the USM group was significantly decreased compared with those in the control group and/or QUE group. Plasma ALT level in USM group was significantly reduced compared to control group, but no significant change was observed in the level of plasma BUN and creatinine. The AI and the CRF were also calculated (**Fig. 6**). AI and CRF levels in the USM group were significantly decreased compared to the control or QUE groups. In present study, it was founded that USM exerted hypolipidemic effects by decreasing epididymal and abdominal adipose tissues' weight, and levels of AI and CRFs.

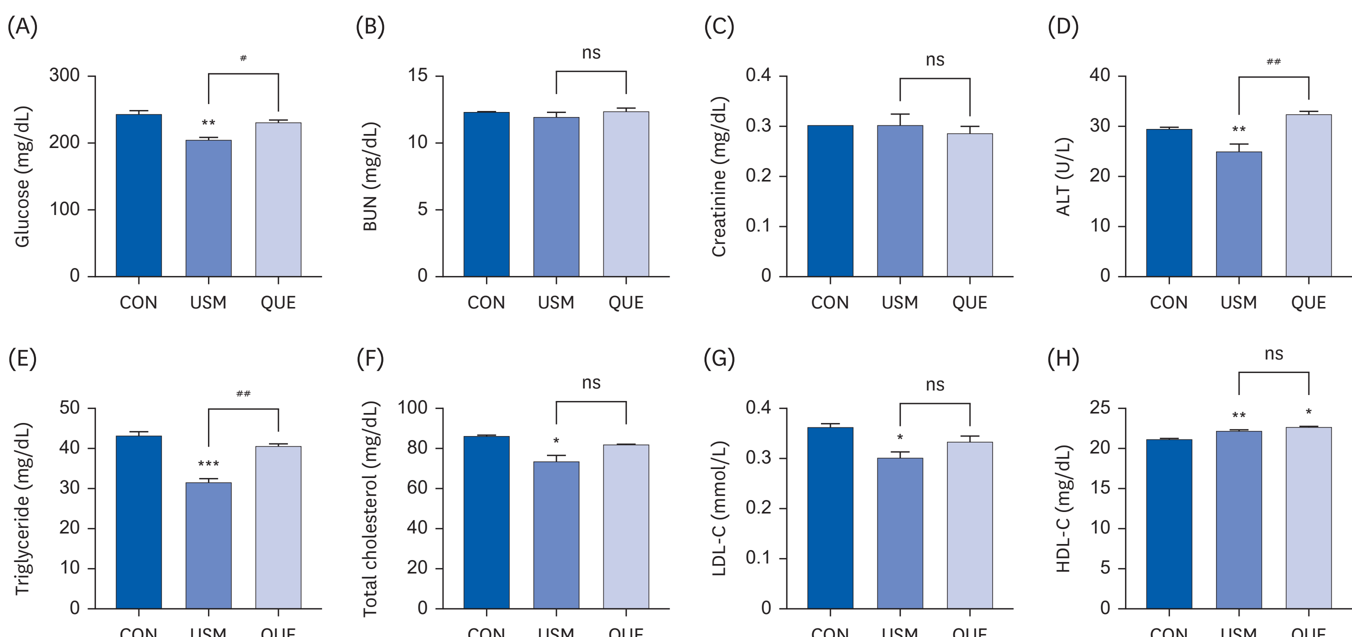

Fig. 5. Effect of unsaponifiable matter (10 mg/kg) from okra seed on glucose level (A), BUN (B), creatin (C), ALT (D), TG (E), TC (F), LDL-C (G) and HDL-C (H). The results are expressed as means ± SD. Quercetin was used as positive control sample (30 mg quercetin/kg).
BUN, blood urea nitrogen; ALT, alanine transaminase; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CON, control; USM, unsaponifiable matter; QUE, quercetin; ns, not significant; ns, not significant.
Asterisk (*) indicates a significant difference versus the control group ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Pound (#) indicates a significant difference between USM and QUE groups ($^{\#\#}P < 0.01$).

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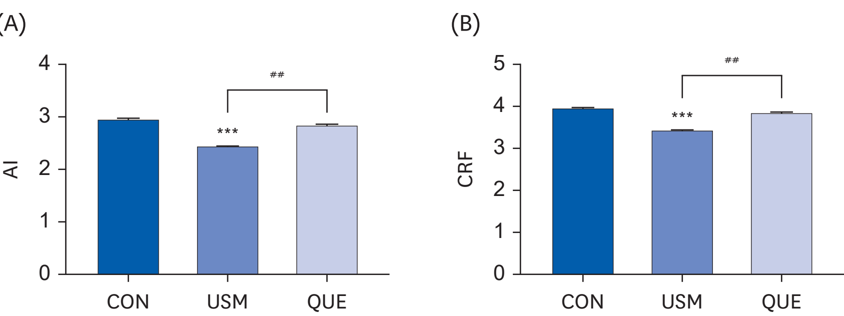

Fig. 6. Effect of unsaponifiable matter (10 mg/kg) from okra seed on AI (A) and the CRF (B) of plasma in GK rat for 5 wk. AI = (TC − HDL-C)/HDL-C; CRF = TC/HDL-C. The results are expressed as means ± SD. Quercetin was used as positive control sample (30 mg quercetin/kg).
AI, atherogenic index; CRF, cardiac risk factor; GK, Goto-Kakizaki; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; CON, control; USM, unsaponifiable matter; QUE, quercetin.
Asterisk (*) indicates a significant difference versus the control group (***P < 0.001). Pound (#) indicates a significant difference between USM and QUE groups (##P < 0.01).

DISCUSSION

It is well documented that phytosterols decrease LDL-C levels in blood serum [25]. One of the possible hypocholesterolemic action of phytosterol is the physical competition between phytosterols and cholesterol for incorporation into micelles, which compromises cholesterol absorption [26]. In addition, phytosterol has antidiabetic property, for example, aloe vera–derived phytosterols ameliorated hyperglycemia in treated db/db type 2 diabetic mice [27]. Since these reasons, plant resources containing phytosterols have drawn attention as a therapeutic dietary option to reduce the risk of metabolic diseases. Our USM from okra seed contained higher amounts of vitamin E (31.16 mg/g) compared to USM from rice bran (23.51 mg/g) or wheat bran (24.90 mg/g) [28,29]. Tocopherols are regarded as intracellular antioxidants due to their activity in inhibiting the peroxidation of polyunsaturated fatty acids in biological membranes. Although α-tocopherol is the most active form in the vitamin E group *in vivo*, hypocholesterolemic, antitumor, neuroprotective, and antioxidant activities of tocotrienols have recently received much attention [30]. Moreover, α-tocopherol supplementation in patients with non-insulin-dependent diabetes mellitus improves insulin action [31]. Therefore, our data imply that USM from okra seeds containing these active compounds may contribute to attenuating hyperlipidemia.

Previously, it was reported that β-sitosterol-mediated enhancement of glucose uptake and reduction of triglycerides and cholesterol in myotube cells is predominantly accomplished by AMP-activated protein kinase activation [32]. Also, a plant sterol from *Adathoda vasica* promoted glucose uptake under insulin resistant condition through the PI3K dependent pathway [33]. Moreover, the long-term use of supplementation of vitamin E showed the decrease of blood glucose via modulation of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase [34]. Among the diabetes-related signaling molecules, PPARγ is involved in lipid and glucose metabolisms. Anti-diabetic natural compounds such as artepillin C and quercetin were shown to increase glucose uptake by targeting the PPARγ, as well as chlorogenic acid and berberine, which stimulated glucose uptake by enhancing GLUT4 and PPARγ gene activity [35]. Therefore, our results suggest that USM from okra seed increases the glucose uptake activity in 3T3-L1 adipocytes via the enhancement of PPARγ and GLUT4.

Yeon *et al.* [18] reported that the rice bran USM contained considerable amounts of vitamin E and phytosterols (23.51 mg of vitamin E, 52.57 mg of campesterol and 190.27 mg of β-sitosterol per one gram of USM. Also, these components have various health beneficial properties such as antioxidant, anti-inflammatory properties and hypocholesterolemic activity [36,37]. In this study, USM from okra seed contained higher amount of vitamin E (31.16 mg/g USM) and phytosterols (394.13 mg/g USM) compared to the USM from rice bran. In diabetic rats, USM significantly reduced the increases in blood glucose and liver and adipose tissue weight, without affecting body weight. Thus, our results suggest that USM from okra seed could suppress blood glucose and fat accumulation in the liver, abdominal and epididymal adipose tissues in diabetic rats.

The ALT level reflects the degree of damage to hepatocytes and an increasing ALT in the blood represents the degree of liver damage [38]. The increased level of plasma BUN and creatinine indicates injured renal function in the diabetic animals [39]. Thus, these results indicate that USM from okra seed did not show the hepatotoxicity and kidney toxicity in diabetic rats. HDL-C has been reported to be effective that improves arteriosclerosis and vascular diseases [40]. In contrast, LCL-C is known to be an important risk factor for the occurrence of arteriosclerosis and cardiovascular diseases, because it can cause arteriosclerosis by accumulating cholesterol in the walls of the arteries [41,42]. High levels of AI and CRF are usually associated with the risk of development of arteriosclerosis and circulatory disorder [24]. It has been well studied that phytosterols and vitamin E decrease the LDL-C. In recent years, a rising number of anti-diabetic studies have demonstrated phytosterols to be helpful *in vivo* and *in vitro*, and it is becoming more popular as a natural treatment [43,44]. Vitamin E attenuated elevated plasma lipid profile and reduced plasma levels of oxidized-LDL-C in diabetic rats [45]. Moreover, previous studies showed that the USM from plant resources showed the anti-obesity and hypolipidemic activities in obese mice *in vivo* [21]. Therefore, these results suggest that the hypoglycemic and hypolipidemic activities of USM from okra seed in diabetic rats can be related to its high levels of phytosterols and tocols.

In conclusion, USM from okra seed decreased blood glucose and adipose tissues weight in diabetes rat. USM also ameliorated lipid metabolic parameters such as levels of TC, TG, and LDL-C in serum samples compared to control. These results showed that USM enhanced anti-diabetic properties in *in vivo* systems. Taken together, these results demonstrated that USM from okra seeds possesses anti-obesity and hypolipidemic properties. These results provide a new insight into enhancing the functional properties of okra seed.

REFERENCES

1. Devalaraja S, Jain S, Yadav H. Exotic fruits as therapeutic complements for diabetes, obesity and metabolic syndrome. Food Res Int 2011;44:1856-65. PUBMED | CROSSREF
2. Lois K, Kumar S. Obesity and diabetes. Endocrinol Nutr 2009;56 Suppl 4:38-42. PUBMED | CROSSREF
3. Nawrocki AR, Scherer PE. Keynote review: the adipocyte as a drug discovery target. Drug Discov Today 2005;10:1219-30. PUBMED | CROSSREF
4. Vázquez-Vela ME, Torres N, Tovar AR. White adipose tissue as endocrine organ and its role in obesity. Arch Med Res 2008;39:715-28. PUBMED | CROSSREF
5. Booth A, Magnuson A, Fouts J, Foster MT. Adipose tissue: an endocrine organ playing a role in metabolic regulation. Horm Mol Biol Clin Investig 2016;26:25-42. PUBMED | CROSSREF
6. Chukwuma CI, Mopuri R, Nagiah S, Chuturgoon AA, Islam MS. Erythritol reduces small intestinal

glucose absorption, increases muscle glucose uptake, improves glucose metabolic enzymes activities and increases expression of Glut-4 and IRS-1 in type 2 diabetic rats. Eur J Nutr 2018;57:2431-44. PUBMED | CROSSREF

7. Zhu K, Nie S, Li C, Lin S, Xing M, Li W, Gong D, Xie M. A newly identified polysaccharide from *Ganoderma atrum* attenuates hyperglycemia and hyperlipidemia. Int J Biol Macromol 2013;57:142-50. PUBMED | CROSSREF
8. Parikh NH, Parikh PK, Kothari C. Indigenous plant medicines for health care: treatment of diabetes mellitus and hyperlipidemia. Chin J Nat Med 2014;12:335-44. PUBMED | CROSSREF
9. Shaik Mohamed Sayed UF, Moshawih S, Goh HP, Kifli N, Gupta G, Singh SK, Chellappan DK, Dua K, Hermansyah A, Ser HL, et al. Natural products as novel anti-obesity agents: insights into mechanisms of action and potential for therapeutic management. Front Pharmacol 2023;14:1182937. PUBMED | CROSSREF
10. Karri S, Sharma S, Hatware K, Patil K. Natural anti-obesity agents and their therapeutic role in management of obesity: a future trend perspective. Biomed Pharmacother 2019;110:224-38. PUBMED | CROSSREF
11. Islam MT. Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*): a literature-based review. Phytother Res 2019;33:72-80. PUBMED | CROSSREF
12. Elkhalifa AE, Alshammari E, Adnan M, Alcantara JC, Awadelkareem AM, Eltoum NE, Mehmood K, Panda BP, Ashraf SA. Okra (*Abelmoschus esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. Molecules 2021;26:696. PUBMED | CROSSREF
13. Afinisha Deepam LS, Arumughan C. Effect of saponification on composition of unsaponifiable matter in rice bran oil. J Oleo Sci 2012;61:241-7. PUBMED | CROSSREF
14. Sharma RD, Rukmini C. Hypocholesterolemic activity of unsaponifiable matter of rice bran oil. Indian J Med Res 1987;85:278-81. PUBMED
15. Lee H, Sung J, Kim Y, Jeong HS, Lee J. Protective effects of unsaponifiable matter from perilla seed meal on UVB-induced damages and the underlying mechanisms in human skin fibroblasts. Antioxidants (Basel) 2019;8:644. PUBMED | CROSSREF
16. Seo D, Jeon A, Shin EC, Lee J, Hwang IG, Kim Y. Evaluation of bioactive compounds contents and biological activities of okra seeds oils. J Korean Soc Food Cult 2020;35:392-9.
17. Shin EC, Pegg RB, Phillips RD, Eitenmiller RR. Commercial peanut (*Arachis hypogaea* L.) cultivars in the United States: phytosterol composition. J Agric Food Chem 2010;58:9137-46. PUBMED | CROSSREF
18. Yeon J, Lee J, Kim Y. Comparison of phytochemical contents and cytoprotective effects of different rice bran extracts from *Indica* and *Japonica* rice cultivars. Prev Nutr Food Sci 2020;25:432-9. PUBMED | CROSSREF
19. Kim Y, Park Y, Namkoong S, Lee J. Esculetin inhibits the inflammatory response by inducing heme oxygenase-1 in cocultured macrophages and adipocytes. Food Funct 2014;5:2371-7. PUBMED | CROSSREF
20. Kim Y, Lee J. Effect of (-)-epigallocatechin-3-gallate on anti-inflammatory response via heme oxygenase-1 induction during adipocyte-macrophage interactions. Food Sci Biotechnol 2016;25:1767-73. PUBMED | CROSSREF
21. Ham H, Sung J, Lee J. Effect of rice bran unsaponifiables on high-fat diet-induced obesity in mice. J Food Biochem 2015;39:673-81. CROSSREF
22. Zhao L, Zhang Q, Ma W, Tian F, Shen H, Zhou M. A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota. Food Funct 2017;8:4644-56. PUBMED | CROSSREF
23. Peng J, Li Q, Li K, Zhu L, Lin X, Lin X, Shen Q, Li G, Xie X. Quercetin improves glucose and lipid metabolism of diabetic rats: involvement of Akt signaling and SIRT1. J Diabetes Res 2017;2017:3417306. PUBMED | CROSSREF
24. Kang NH, Lee WK, Yi BR, Lee HR, Park MA, Park SK, Park HK, Choi KC. Risk of cardiovascular disease is suppressed by dietary supplementation with protamine and chitooligosaccharide in Sprague-Dawley rats. Mol Med Rep 2013;7:127-33. PUBMED | CROSSREF
25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97. PUBMED | CROSSREF
26. de Jong A, Plat J, Mensink RP. Metabolic effects of plant sterols and stanols (Review). J Nutr Biochem 2003;14:362-9. PUBMED | CROSSREF
27. Tanaka M, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, Toida T, Hayasawa H, Takase M, Inagaki M, et al. Identification of five phytosterols from Aloe vera gel as anti-diabetic compounds. Biol Pharm Bull 2006;29:1418-22. PUBMED | CROSSREF

28. Ham H, Yoon SW, Kim IH, Kwak J, Lee JS, Jeong HS, Lee J. Protective effects of unsaponifiable matter from rice bran on oxidative damage by modulating antioxidant enzyme activities in HepG2 cells. Lebensm Wiss Technol 2015;61:602-8. CROSSREF
29. An M, Heo H, Park J, Jeong HS, Kim Y, Lee J. Unsaponifiable matter from wheat bran cultivated in Korea inhibits hepatic lipogenesis by activating AMPK pathway. Foods 2023;12:4016. PUBMED | CROSSREF
30. Comitato R, Ambra R, Virgili F. Tocotrienols: a family of molecules with specific biological activities. Antioxidants (Basel) 2017;6:93. PUBMED | CROSSREF
31. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am J Clin Nutr 1993;57:650-6. PUBMED | CROSSREF
32. Hwang SL, Kim HN, Jung HH, Kim JE, Choi DK, Hur JM, Lee JY, Song H, Song KS, Huh TL. Beneficial effects of beta-sitosterol on glucose and lipid metabolism in L6 myotube cells are mediated by AMP-activated protein kinase. Biochem Biophys Res Commun 2008;377:1253-8. PUBMED | CROSSREF
33. Sujatha S, Anand S, Sangeetha K, Shilpa K, Lakshmi J, Balakrishnan A, Lakshmi B. Biological evaluation of (3β)-STIGMAST-5-EN-3-OL as potent anti-diabetic agent in regulating glucose transport using *in vitro* model. Int J Diabetes Mellit 2010;2:101-9. CROSSREF
34. Balbi ME, Tonin FS, Mendes AM, Borba HH, Wiens A, Fernandez-Llimos F, Pontarolo R. Antioxidant effects of vitamins in type 2 diabetes: a meta-analysis of randomized controlled trials. Diabetol Metab Syndr 2018;10:18. PUBMED | CROSSREF
35. Prabhakar PK, Doble M. Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. Phytomedicine 2009;16:1119-26. PUBMED | CROSSREF
36. Wilson TA, Nicolosi RJ, Woolfrey B, Kritchevsky D. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. J Nutr Biochem 2007;18:105-12. PUBMED | CROSSREF
37. Zhu Y, Sang S. Phytochemicals in whole grain wheat and their health-promoting effects. Mol Nutr Food Res 2017;61:1600852. PUBMED | CROSSREF
38. Goldberg DM, Watts C. Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterology 1965;49:256-61. PUBMED | CROSSREF
39. Asagba SO, Kadiri HE, Ezedom T. Biochemical changes in diabetic rats treated with ethanolic extract of *Chrysophyllum albidum* fruit-skin. J Basic Appl Zool 2019;80:42. CROSSREF
40. Kannel WB, Dawber TR, Friedman GD, Glennon WE, McNamara PM. Risk factors in coronary heart disease: an evaluation of several serum lipids as predictors of coronary heart disease; the Framingham study. Ann Intern Med 1964;61:888-99. PUBMED | CROSSREF
41. Gordon T, Kannel WB, Castelli WP, Dawber TR. Lipoproteins, cardiovascular disease, and death: the Framingham study. Arch Intern Med 1981;141:1128-31. PUBMED | CROSSREF
42. Stamler J, Wentworth D, Neaton JD. Prevalence and prognostic significance of hypercholesterolemia in men with hypertension. Prospective data on the primary screenees of the multiple risk factor intervention trial. Am J Med 1986;80:33-9. PUBMED | CROSSREF
43. Babu S, Krishnan M, Rajagopal P, Periyasamy V, Veeraraghavan V, Govindan R, Jayaraman S. Beta-sitosterol attenuates insulin resistance in adipose tissue via IRS-1/Akt mediated insulin signaling in high fat diet and sucrose induced type-2 diabetic rats. Eur J Pharmacol 2020;873:173004. PUBMED | CROSSREF
44. Krishnan M, Babu S, Rajagopal P, Nazar SP, Chinnaiyan M, Jayaraman S. Effect of β-sitosterol on insulin receptor, glucose transporter 4 protein expression and glucose oxidation in the gastrocnemius muscle of high fat diet induced type -2 diabetic experimental rats. Indian J Pharm Educ Res 2021;55:s479-91. CROSSREF
45. Shirpoor A, Norouzi L, Nemati S, Khadem Ansari MH. Protective effect of vitamin E against diabetes-induced oxidized LDL and aorta cell wall proliferation in rat. Iran Biomed J 2015;19:117-23. PUBMED

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