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THE POTENTIAL OF SUNFLOWER SEED BISCUITS IN LOWERING BLOOD GLUCOSE AND MALONDIALDEHYDE LEVEL IN TYPE 2 DIABETES MELLITUS RAT MODELS

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is marked by early hyperglycemic symptoms and concurrent insulin resistance, leading to insulin secretion dysregulation. This resistance correlates with heightened Reactive Oxygen Species (ROS) level and reduced malondialdehyde. T2DM elevates malondialdehyde, necessitating antioxidant-rich interventions. Sunflower seed biscuits serve as a rich source of enzymatic antioxidants. The primary objective of this investigation was to substantiate the capacity of sunflower seed biscuits to ameliorate blood glucose and malondialdehyde level in T2DM-afflicted rats. Sunflower seed biscuits were investigated for their impact on blood glucose and malondialdehyde in 24 male Wistar rats. Rats were divided into four groups: diabetes rats (K-), simvastatin-treated (K+), receiving 0.72 g sunflower seed biscuits per rat body weight (X1), and 1.44 g (X2). Administered for 28 days via oral gavage, T2DM was induced through a high-fat diet and streptozotocin. Results showed significant blood glucose reduction in treatment groups (X1: 3.99 ng/mL, X2: 2.89 ng/mL) vs. controls (9.8 ng/mL), with statistical significance ($p<0.05$). Sunflower seed biscuits effectively lowered blood glucose and malondialdehyde in T2DM rats. The X2 group exhibited superior efficacy in reducing both parameters. Thus, sunflower seed biscuits present promise as a viable dietary option for T2DM management.

Keywords: Sunflower seed, diabetes mellitus, blood glucose, malondialdehyde

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a pathological condition delineated by the initial manifestation of hyperglycemic symptoms and a concomitant perturbation in insulin secretion, colloquially referred to as insulin resistance (Perkeni, 2015). The incidence of T2DM frequently correlates with obesity, specifically the augmentation of body weight, which can engender a decrement in the functionality of pancreatic β cells (Al-Sulaiti, et al., 2019). This decrement in insulin sensitivity ultimately precipitates the occurrence of hyperglycemia (Xu, et al., 2019). Hyperglycemic states have the propensity to augment oxidative stress conditions, marked by heightened production of Reactive Oxygen Species (ROS) and serving as a catalyst for elevated free radical generation. The genesis of free radicals within the milieu of T2DM is primarily attributed to the phenomenon of glucose undergoing an auto-oxidative process that surpasses the rate of endogenous antioxidant formation within the organism (Asmat, et al., 2016).

Pathological states that foster an upsurge in free radical level, devoid of commensurate endogenous and exogenous antioxidant reservoirs, have the potential to exacerbate oxidative stress (Asmat, et al., 2016). The proliferation of Reactive Oxygen Species (ROS) within the organism can be reinstated to homeostatic level through the administration of both enzymatic and non-enzymatic antioxidants (Jamuna and Mythili, 2014). Non-enzymatic antioxidants, derived from natural dietary constituents, have demonstrated efficacy in ameliorating the clinical manifestations of diabetes mellitus (Kinasih, et al., 2020; Kurniawan, et al., 2020).

Sunflower seeds represent a natural dietary component that has yet to be fully leveraged. Empirical investigations underscore the substantial presence of essential components within sunflower seeds, including vitamin E, linoleic acid, β-sitosterol, and phenolics, rendering them a valuable reservoir of enzymatic antioxidants (Kiczorowska, et al., 2019; Saini and Sharma, 2013). Sunflower seeds exhibit a notable

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phenolic content, quantified at 977.0 mg per 10 gram (Zoumpoulakis, et al., 2017). Beyond their enzymatic antioxidant potential, sunflower seeds have been ascribed additional roles, encompassing anti-inflammatory, antioxidant, and anti-diabetic properties (Zoumpoulakis, et al., 2017).

Sunflower seed biscuits are categorized as functional foods, distinguished by their prolonged shelf stability and enriched complexity in nutrients and bioactive compounds in contrast to unprocessed sunflower seeds (Leverrier et al., 2019). The biscuits' composition comprises 70% sunflower seeds and 30% other constituents. The dosage variants of sunflower seed biscuits administered in this investigation were 0.72 g/day and 1.44 g/day, as previously established in the literature, aimed at attenuating the pro-inflammatory response in diabetic Wistar rats (Mahirdini and Afifah, 2016). This approach aligns with prior research findings (Leverrier et al., 2019; Saini and Sharma, 2013), underscoring its validity in mitigating diabetic-related inflammation.

In accordance with the aforementioned elucidation, the principal objective of this investigative endeavor is to ascertain the therapeutic potential of sunflower seed biscuits (SSB) in mitigating blood glucose level and malondialdehyde concentrations within the context of Type 2 Diabetes Mellitus (T2DM) in Wistar rat models.

METHODS

This study adheres to a rigorous true experimental design, employing a Pre and Post Test with Control Group Design. The analysis of biscuit nutritional composition, encompassing parameters such as energy, protein, fat, carbohydrate, and antioxidant content, was conducted at Chem-Mix Pratama Laboratory in Yogyakarta. Experimental animal husbandry and testing procedures were meticulously executed at the Laboratory of Center for Food and Nutrition Studies (*Pusat Studi Pangan dan Gizi* - PSPG), Universitas Gadjah Mada, Yogyakarta, spanning the period from November 2020 to January 2021. Notably, this research initiative secured the requisite ethical clearance and authorization from the Health Research Ethics Commission at Diponegoro University, Dr. Kariadi, bearing registration number 113/EC/H/FK-UNDIP/XI/2020.

The determination of the sample size for this experiment adhered to the guidelines established by the World Health Organization (WHO, 2000), specifying a minimum requirement of 5 mice per treatment group for experimental animals. A cohort comprising 24 male Wistar rats, aged between 8 and 12 weeks, and exhibiting body weights ranging from 150 to 200 gram, was procured from the Laboratory of the Center for Food and Nutrition Studies (PSPG), Universitas Gadjah Mada, Yogyakarta, in accordance with Marques et al. (2016). The constituents requisite for biscuit formulation were sourced from a confectionery establishment in Semarang. Streptozotocin (STZ) and Nicotinamide (NA) were acquired from Nacalai Tesque, Japan. The quantification of blood glucose level was executed utilizing the GOD-PAP method, while the measurement of malondialdehyde level was performed employing the ELISA technique in the course of this investigation.

The baseline rat nutrition regimen consisted of Comfeed II, comprising 12% water, 15% crude protein, 3–7% crude lipid, 6% crude fiber, 7% ash, 0.9–1.1% calcium, and 0.6–0.9% phosphorus, administered at a dosage of 20 gram per rat per day. Subsequently, the high-fat feed administered to induce Type 2 Diabetes Mellitus (T2DM) in the rats was comprised of 80% standard feed, 20% pork oil, and 1.5% additional cholesterol, administered at 20 gram per rat per day for a duration of 14 days, followed by ad libitum access to distilled water. Essential experimental equipment encompassed cages, rat feeding troughs, rat drinking bottles, digital scales, and blood sampling apparatus, including micro hematocrit and syringe probes, all procured from the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta.

In this experimental study, the administration of sunflower seed biscuits was meticulously calibrated to ensure equivalency between human and rat dosage. The standard serving size for human snacks, consisted of 40 gram of biscuits, was employed as the benchmark. To establish the corresponding dosage for rats, the sunflower seed content in 100 gram of biscuits was determined

through meticulous calculation. For Dose 1, an allocation of 0.72 gram of biscuits was employed, which correspondingly contained 0.504 gram of sunflower seeds. Within this dosage, the provision of vitamin E amounted to 12.68 mg. In contrast, Dose 2 constituted a 1.44 gram allocation of biscuits, yielding 1.008 gram of sunflower seeds. Within this dosage regimen, the delivery of vitamin E escalated to 25.36 mg. This precise dosing methodology ensures a methodical investigation into the potential effects of sunflower seeds and vitamin E, thereby contributing to the scientific understanding of their impact on rat physiology. Such meticulous dosage calibration is fundamental in the realm of medical research, as it permits accurate interpretation of outcomes and facilitates the derivation of meaningful insights into potential therapeutic or nutritional interventions.

The sunflower seed biscuit preparation commences with meticulous selection of 70 g of dry sunflower seeds and 10 g of oats, both meticulously ground to a homogenous texture via a blender. Subsequently, amalgamate this mixture with 10 g of eggs, 5 g of low-calorie granulated sugar, and 5 g of margarine until uniform. The dough is then flattened into 10 g portions and baked at 130°C for precisely 15 minutes. This meticulous culinary process yields delectable sunflower seed biscuits.

Table 1. Feed Composition of Intervention Rat

Ingredient	Composition (%)
Sunflower seed	70
Oat	10
Chicken egg	10
Low caloric sugar	5
Margarine	5

The induction of type 2 diabetes mellitus (T2DM) in murine models necessitates a meticulously orchestrated protocol. In this endeavor, 24 male Wistar rats were subjected to a systematic regimen. Over the course of one week, these rats were individually housed, receiving a standard daily diet of 20 gram and ad libitum access to distilled water. Ambient temperature was maintained within the range of 28–32°C, complemented by a 12-hours light cycle spanning from 06:00 to 18:00. Rigorous daily monitoring encompassed food consumption quantification and routine cage sanitation. Prior to commence T2DM induction, it was imperative to establish baseline parameters for each subject. Blood samples were extracted via the retroorbital plexus to ascertain uniform blood glucose and malondialdehyde level. This preliminary screening ensured the health status of all mice at the inception of the study. The subsequent phase of the experiment involved the induction of T2DM in the murine cohort. This was achieved through a 14-days dietary intervention, during which high-fat food was administered at a rate of 20 gram per rat per day (Guo, et al., 2018). Subsequent to Niconitamide (NA) induction at 110 mg/kg intraperitoneally, Streptozotocin (STZ) was administered intraperitoneally at 45 mg/kg, 15 minutes later. (Ghasemi, et al., 2014; Qasem, et al., 2018). Prior to streptozotocin (STZ) induction, mice were subjected to a fasting regimen. Following 3 days of STZ induction, mice underwent an additional 8-10 hours fast, after which 2 mL of blood was sampled from the retroorbital plexus to assess blood glucose level. Malondialdehyde served as a baseline comparator prior to initiating the intervention involving SFS biscuits. The diagnostic threshold for type 2 diabetes mellitus was established at fasting blood glucose level ≥200 g (Li, et al., 2015).

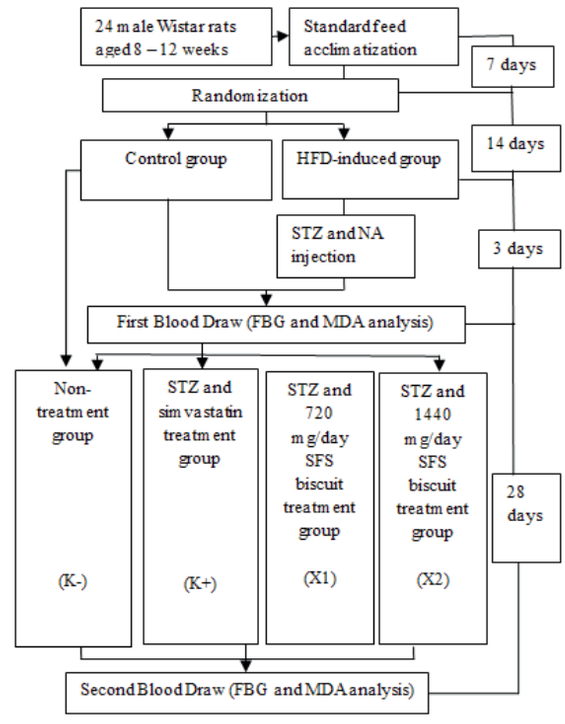

Figure 1. Research Flowchart

In this experimental study, twenty-four rats were stratified into four distinct groups: T2DM rats without any therapeutic intervention (Group K-), T2DM rats administered a daily dosage of 0.18 mg/rat weight/day of simvastatin (Group K+), T2DM rats receiving a daily dose of 0.72 g/rat weight/day of sunflower seed biscuits (Group X1), and T2DM rats subjected to a daily dose of 1.44 g/rat weight/day of sunflower seed biscuits (Group X2). The sunflower seed biscuits were meticulously crushed and transformed into a daily solution, which was freshly prepared each morning. Subsequently, this solution was administered to the T2DM rats via sonde, once daily, in the morning. The intervention involving the sunflower seed biscuit solution in T2DM rats spanned a duration of 28 days, with the rats being concurrently provided with standard food and water throughout the experimental period (Kinasih, *et al*. 2020). Upon study completion, blood samples were collected for conclusive assessment of blood glucose and malondialdehyde concentrations following the intervention.

In this experimental study, data underwent normality assessment via Shapiro-Wilk test. Normal distribution confirmed, enabling the use of paired T test for pre-post comparison. Group distinctions analyzed via *One-Way ANOVA*, followed by *Bonferroni post hoc* tests. *Spearman Rank correlation* explored relationships with $p<0.05$ significance level.

RESULTS AND DISCUSSION

Rats Weights during Experiment

Table 2 illustrates the weight gain observed in the experimental animal groups during the SFS biscuit administration period. Significant disparities emerged between the pre-test and post-test measurements across all four groups. In the K(+) group, X1, and (K-), a notable decline occurred due to the absence of simvastatin and SFS biscuit intervention in the context of established T2DM. This exacerbated the T2DM condition, intensifying oxidative stress level without antioxidant supplementation. Consequently, these mice exhibited decreased appetite, leading to a decline in body weight (Tabatabaei-Malazy, et al. 2017). Pre-intervention, groups exhibited similar mean body weights; post-SFS biscuit administration, all groups showed significant body weight disparities ($p<0.05$).

Injection of streptozotocin in group K(-) mice led to reduced weight loss, attributed to heightened fat decomposition and DNA synthesis inhibition in mammalian enzymes, triggering apoptosis (He, et al., 2015). Insulin resistance can be a factor that induce weight loss in mice by impairing the inhibitory effect of insulin on lipolysis via the cAMP pathway, leading to reduced PKA activation. In the K(-) group, mice exhibited a 21.5% decrease in appetite, translating to an average daily intake reduction of 2 gram. This diminished food consumption contributed to the observed decline in body weight during the study (Fauza, et al., 2019). STZ-induced enzyme inhibition triggers cellular apoptosis, contributing to weight loss in T2DM-afflicted mice. (Nagarchi, et al. 2015).

The observed increase in rat body weight in the X2 group closely resembled that in the K(+) group. This suggests that SFS biscuits exhibit comparable efficacy to simvastatin in mitigating oxidative stress, thereby enhancing food intake and influencing body weight parameters. Notably, the

Table 2. Rat Body Weights *Pre* and *Post* Treatment

Treatment Group	Rat Body Weight (gram)			
	Pre	***Post***	***p***	Δ
K(-)	202.5±4.7	184.3±4.2	<0.001	18.2±1.5
K(+)	205.2±3.1	231.0±3.3	<0.001	25.8±1.9
X1	203.2±5.2	221.3±4.6	<0.001	18.2±1.2
X2	206.2±3.9	232.2±4.5	<0.001	26.0±1.7
p^1	*0.435	<0.001		<0.001

Note: *p*= paired T test; p^1 = *One-Way ANOVA* test; *= *Kruskal Wallis* test; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastasin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples

X2 group demonstrated the highest weight gain, likely attributed to the substantial protein content (22.7 gram per 100 gram) in BBM biscuits, which enhanced palatability and appetite in the mice.

Post-Treatment Changes in Blood Glucose and Malondialdehyde Level

The paired T-test analysis presented in Tables 3 and 4 demonstrated a noteworthy decline in blood glucose and malondialdehyde level following the intervention. Additionally, Table 5 illustrated a significant correlation between blood glucose and malondialdehyde after treatment, using doses of 0.72 g/rat body weight/day and 1.44 g/rat body weight/day ($p<0.05$). In this experimental context, SFS biscuits displayed a statistically proven capacity to substantially reduce blood glucose level in T2DM mice. Notably, the X2 group exhibited a markedly superior reduction in blood glucose level compared to the group administered simvastatin K(+). Both X1 and X2 doses displayed significant efficacy in reducing blood glucose level. Remarkably, the effectiveness of the X2 group in reducing blood glucose level rivalled that of simvastatin in T2DM mice. Further analysis, utilizing Bonferroni's post hoc test ($p<0.05$), revealed significant differences between treatments in both X1 and X2 (Table 2).

The most significant reduction in blood glucose level was observed within group X2, administered a daily dose of 1.44 g of biscuits, resulting in a value of 95.2 ± 1.9 mg/dL in comparison to group X1. This outcome can be attributed to the heightened antioxidant content within this dosage, along with its rich vitamin E and phenolic composition. Earlier investigations have identified various antioxidant components in sunflower seeds, including phenolic compounds and vitamin E, as reported by Widia et al. (2011) and Kiczorowska et al. (2019). These constituents are recognized for their potential in mitigating blood glucose level. Moreover, the active substances present in pancreatic β cells and the antioxidants found in SFS biscuits contribute to the reduction of malondialdehyde level in T2DM mice, as illustrated in Table 3.

A substantial reduction in malondialdehyde (MDA) level was observed in both treatment cohorts. Mice administered SFS biscuits at a dosage of 1.44 g per rat per day exhibited MDA level closely mirroring those in the group receiving

Table 3. Blood Glucose Level *Pre* and *Post*-treatment

Treatment Group	Blood Glucose Level (mg/dl)			
	Pre	***Post***	***p***	Δ
K(-)	270.1±2.1	272.3±2.2	0.001^{abc}	2.1±0.8
K(+)	268.2±1.9	89.7±3.5	$<0.001^{b}$	178.5±4.4
X1	269.8±2.2	135.9±3.8	$<0.001^{ac}$	133.8±5.5
X2	268.9±2.1	95.2±1.9	$<0.001^{b}$	173.8±3.1
p^1	0.379	<0.001		<0.001

Note: *p*= paired T test; p^1 = *One-Way ANOVA* test; $^{a}p<0.05$ *post hoc Bonferroni* test with group K(+); $^{b}p<0.05$ *post hoc Bonferroni* test with group X1; $^{c}p<0.05$ *post hoc Bonferroni* test with group X2; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastasin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples

Table 4. Malondialdehida Level *Pre* dan *Post*-Treatment

Treatment Group	Malondialdehida (ng/dl)			
	Pre	***Post***	***p***	Δ
K(-)	9.7±0.6	9.8±0.5	0.056^{abc}	0.1±0.1
K(+)	9.8±0.4	1.8±0.2	$<0.001^{bc}$	8.0±0.5
X1	9.8±0.4	4.0±0.2	$<0.001^{ac}$	5.8±0.4
X2	9.7±0.3	2.9±0.2	$<0.001^{ab}$	6.9±0.5
p^1	0.908	<0.001		<0,001

Note: *p*= paired T test; p^1 = *One-Way ANOVA* test; $^{a}p<0.05$ *post hoc Bonferroni* test with group K(+); $^{b}p<0.05$ *post hoc Bonferroni* test with group X1; $^{c}p<0.05$ *post hoc Bonferroni* test with group X2; K(-)= T2DM + non-treatment; K(+)= T2DM + simvastasin; X1= T2DM + sunflower seed biscuit 0.72 g/rat BW/day; X2= T2DM + sunflower seed biscuits 1.44 g/rat BW/day; n= 24 samples.

0.18 mg of simvastatin daily. This investigation underscores the potential equivalence between SFS biscuit-based nutritional intervention and simvastatin pharmaceutical therapy. Notably, a significant contrast emerged between treatment X1 and X2, with the latter yielding the most pronounced MDA reduction at 2.9 ± 0.2 ng/mL, surpassing the former. The administered dosage aligns with established precedents from prior studies, affirming its efficacy in mitigating inflammatory responses in diabetic murine models (Saboori, et al., 2015; Leverrier, et al., 2019; Saini dan Sharma, 2013). Vitamin E in sunflower seeds serves as an antioxidant, augmenting total antioxidant capacity to thwart free radical formation. Furthermore, it mitigates inflammation by inhibiting interleukin-6 (IL-6) release (Septiani, 2018).

Table 4 illustrates a noteworthy Spearman Rank correlation analysis, revealing a significant ($p<0.05$) association between blood glucose and malondialdehyde level. This study reinforces the inverse relationship: decreased blood glucose corresponds to reduced oxidative stress, as indicated by lower malondialdehyde level.

Table 5. Blood Glucose Level and Malondialdehyde Correlation Pre and Post-Treatment

Variable	Pre		Post	
	r	p	r	p
Blood Glucose and Malondialdehyde	-0.062	0.775	0.991	<0.001

This study demonstrates the substantial unsaturated fat content (35.7g/100g) in SFS biscuits, rich in unsaturated fatty acids. Omega-3's pivotal role includes enhancing insulin sensitivity by mitigating inflammation and elevating adiponectin level (Cheenam, et al., 2019), so this study demonstrates the efficacy of sunflower seeds in reducing blood glucose and malondialdehyde level. Sunflower seed (SFS) biscuits, administered at 0.72 and 1.44 g/weight of mice/day, approximate to 40 and 80 gram or 4 and 8 pieces for humans, prove beneficial as a snack during Type 2 Diabetes Mellitus (T2DM) treatment. Statistical analysis confirms the significant reduction of blood glucose and malondialdehyde level in T2DM mice through SFS biscuit intervention.

CONCLUSIONS

Sunflower seed biscuits exhibit potential in lowering blood glucose and malondialdehyde level in type 2 diabetes mellitus mice, with optimal effects observed at an intervention dose of 1.44 g/rat body weight/day. SFS biscuits, offering 80g or 8 pieces as a snack, are suggested for T2DM patients, potentially lowering blood glucose and malondialdehyde level.

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