Prevention of Inflammation Through Increasing Interleukin-10 (IL-10) in Animal Models of Hyperglycemia

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KEYWORDS

Diabetes mellitus, Hyperglycemia, Inflammation, Interleukin10

ABSTRACT

Persistent high blood sugar levels lead to the malfunction and the decrease in the number of pancreas β cells that produce insulin leads to the initiation of inflammation inside the pancreas. It has been shown that the development of diabetes mellitus is associated with inflammation as well as the stimulation of the intrinsic immunity systems. Increased IL-10 expression plays a role in balancing proinflammatory cytokines that limit tissue damage. This research seeks to evaluate the impact of mangosteen peel extract on preventing inflammation by boosting IL-10 levels in hyperglycemic animal models. The study was conducted as a laboratory experiment, using a distinct pretest-posttest control group design that is randomly. Negative control (KN), positive control with STZ (K1), STZ and CMC-Na (K2), mangosteen peel extract at 200 mg/kg body weight (K3), 400 mg/kg body weight (K4), and 600 mg/kg body weight (K5) were the six groups that were included in the sample. Additionally, the sample was composed of male white rats from the Wistar strain. The treatment was given regularly for 14 days. The research revealed that rats administered with mangosteen peel extract had a significantly greater quantity of IL-10 positive cells, averaging 7.66, in contrast to the uncontrolled group which had an average of 2.44. The number of IL-10-expressing cells differed significantly ($p < 0.05$) across all treatment groups. The group administered with a dosage of 600 mg/kg BW/day of mangosteen peel extract had the greatest count of IL-10-positive cells, demonstrating superior effectiveness compared to the other dosages. This study concludes that different doses of mangosteen peel extract influence the increase in IL-10-positive cells and help prevent inflammation in hyperglycemia, with 600 mg/kg BW/day being the most effective. The findings of this study emphasize the properties of mangosteen peel and indicate that it may have the potential to serve as an alternative therapy for illnesses that are associated with inflammation in humans.

1. Introduction

Diabetes mellitus (DM) may be diagnosed clinically by hyperglycemia, which is defined as increased amounts of glucose in the blood. Persistent hyperglycemia leads to dysfunction and a reduction in the mass of insulin-producing pancreatic β cells. This modification in the functioning and mass of pancreas β-cells leads to histologic abnormalities in the islets of Langerhans, which in turn trigger inflammation inside the pancreas. There is a correlation between the progression of diabetes mellitus and the occurrence of inflammatory activities as well as the activation of the innate immune system. Research on a group of mice shows that inflammatory parameters such as cytokines are strong predictors in the pathogenesis of DM. DM has been categorized as a global disease whose prevalence continues to increase. In 2015, the International Diabetes Federation (IDF) reported that there were 415 million individuals worldwide affected by diabetes mellitus (DM). Indonesia is rated 7th globally, with a population of 10 million people, behind China, India, the United States, Brazil, Russia, and Mexico. According to the IDF, it is anticipated that the incidence of DM will continue to increase by 2040 (Atlas, 2015). The 2018 Riskesdas survey conducted in Indonesia revealed a rise in the incidence of diabetes mellitus (DM) from 6.9% in 2015 to 8.5% in 2018, as described by Milita et al. in 2021. DM is a metabolic illness that impacts the metabolism of carbohydrates, lipids, and proteins. It is characterized by high levels of glucose in the blood, a condition known as hyperglycemia. Persistent elevation of blood glucose levels results in impaired function and a decreased number of pancreatic β cells responsible for insulin production. According to Eguchi et al. (2021), these problems and decreases in β-cell mass indicate alterations in the histology of the pancreatic islets of Langerhans and cause inflammation inside the pancreas. The pathophysiology of diabetes mellitus involves the development of inflammatory processes and the activation of the innate immune system (Lontchi-

Yimagou et al., 2013). According to the study, increasing IL-10 expression plays a role in balancing proinflammatory cytokines, thereby limiting tissue damage. When homeostasis is achieved, inflammation and pain decrease, preparing for tissue repair, stopping damage, and restoring tissue, resulting in healing [27]. The migration of phagocytic cells such as neutrophils and macrophages is a result of inflammatory processes, which is the first immune reaction to an infection, injury, or damage to tissue. Normally, Defense mechanisms are used by the body to restore equilibrium and remove irritants, hence aiding the healing process. However, if immune cells fail to restore balance through these mechanisms, the damage can spread, resulting in chronic conditions and potentially leading to reversible or irreversible cell death (Giri et al., 2018).

Preventing inflammation in diabetes mellitus (DM) involves controlling hyperglycemia, which is a crucial aspect of DM management. Effective management necessitates a multidisciplinary approach, including both non-pharmacological and pharmacological treatments. However, synthetic medications often have a brief duration of effect and might potentially induce unfavorable side effects. Consequently, plant-based compounds are used to treat diabetes (Sunil et al., 2012). Most oral antidiabetic medications can lead to undesirable side effects, as an example, herbal treatment may provide a comparatively safer option by reducing resistance and minimizing harm to organs such as the kidneys. Numerous studies have explored alternative treatments for diabetes mellitus (DM) using vitamins and herbal therapies. In society, the treatment of DM increasingly incorporates traditional medicine, commonly referred to as herbal medicine. This type of medicine includes various ingredients plant-based, animal-derived, or mineral-based, either in extract forms (galenic) or as mixtures of these components that have been used for generations based on traditional practices (Agency, 2004). Research indicates that herbal medicine, specifically mangosteen rind, can assist in regulating glycemic control in individuals diagnosed with diabetes mellitus. Research conducted by Hoffbrand et al. (2019) proved the efficacy of mangosteen peel among the community on seven diabetes patients who consumed mangosteen peel extract for ten days, which was able to reduce blood sugar, where the average sugar level in the 7 patients fell from 205.0 to 119.86 mg/dL. Traditional medicines often contain multiple active compounds, have a slow reaction process in the body, and lack guaranteed safety and hygiene (Kunle et al., 2012). Therefore, it is essential to use active compounds from natural extracts that have undergone separation and purification processes, with dosages established through pre-clinical testing (animal trials) to assess safety. Further research on experimental animals, such as mice, is still necessary. To address these issues, it is crucial to explore methods for preventing inflammation in hyperglycemia to reduce complications in individuals with diabetes mellitus. Research on using mangosteen peel for treating diabetes in humans is limited. Therefore, as a preliminary step, experiments will be conducted to assess the potential benefits of mangosteen peel in preventing inflammation by increasing IL-10. This will involve developing a streptozotocin-induced hyperglycemia model in animals.

2. Methodology

A randomized design with a randomized separated pretest-posttest control group design technique served as the basis for this study, which is a genuine research project. Using the ethical permission number 655/HRECC.FODM/VIII/2022, the research was carried out at the Biochemistry Laboratory from Airlangga University in Surabaya. The study involved two main groups: the control group and the treatment (experiment) group. The positive control group was further divided, receiving either Streptozotocin (STZ) (K1) or the solvent Carboxymethyl Cellulose-Sodium (CMC-Na) (K2). The treatment group with mangosteen peel was split into three sub-groups (K3, K4, and K5), along with a negative control group (KN). Each group in the study was treated differently: the control group (KN) received no treatment, while the initial positive control group (K1) was given Streptozotocin (STZ) at a dose of 45 mg/kg BW/day on the first day to model hyperglycemia. The final positive control group (K2) also received STZ at the same dose on day one, followed by a 0.5% Carboxymethyl Cellulose-Sodium (CMC-Na) solution. The treatment groups were given STZ at the same initial dose and then received mangosteen peel ethanol extract at varying doses: 200 mg/kg BW/day (K3), 400 mg/kg

BW/day (K4), and 600 mg/kg BW/day (K5). The study used male mice aged 3-4 months. Fifty mice underwent an adaptation period of seven days. After adaptation, Streptozotocin (STZ) was administered at a dose of 45 mg/kg BW once on the first day. The therapy was then given orally for 14 days. The mice were randomly assigned to five groups: KN, K1, K2, K3, K4, and K5. At a significance level of $\alpha = 0.05$, the data that were gathered were subjected to statistical analysis using the analysis of variance (ANOVA) test. This was then followed by a post-hoc multiple comparisons test, particularly the LSD test.

3. Results and discussion

In the following table, Table 1, you will see the average and standard deviations of the number of intra-cells that are positive for interleukin-10 (IL-10).

Table 1. Interleukin-10 (IL-10) Mean and Standard Deviation in the Treatment and Control Groups

Group	n	Mean ± SD	Minimum	Maximum
K N	8	$1.30^{a} \pm 0.63$	0.40	2.20
K 1	8	$2.40^{b} \pm 0.75$	1.40	3.80
K2	8	$3.42^{c} \pm 0.68$	2.20	4.40
K3	8	$4.87^{d} \pm 0.70$	4.00	6.00
K4	9	$5.93^{e} \pm 0.80$	4.80	6.80
K5	9	$7.66^{f} \pm 1.48$	5.20	10.20

Information:

KN = The negative control group did not receive any substances from days 1 to 18.

K1 = The initial positive control group received a dosage of 45 mg/kg STZ BB/day 1 from an experimental animal model of hyperglycemia.

K2 = A hyperglycemia animal model was administered 45 mg/kg STZ BW/day on day one and 0.5% CMC-Na solution from days 5 to 18 as the final positive control group.

K3 = In an animal model of diabetes, the treatment group received 45 mg/kg BW/day STZ initially, followed by 200 mg/kg BW/day ethanol extract of mangosteen peel from day 5 to 18.

K4 = In an animal model of hyperglycemia, the treatment group received 45 mg/kg BW/day STZ initially, followed by 400 mg/kg BW/day ethanol extract of mangosteen peel from day 5 to 18.

K5 = To treat hyperglycemia in an animal model, 45 mg/kg BW/day STZ was administered first, followed by 600 mg/kg BW/day ethanol extract of mangosteen peel from day 5 to 18.

Table 1 displays the mean values for each group. The negative control group had a mean of 1.30 ± 0.63, the early positive group had 2.40 ± 0.75, the late positive group had 3.42 ± 0.68, treatment group I had 4.87 ± 0.70, treatment group II had 5.93 ± 0.80, and treatment group III had 7.66 ± 1.48. The average number of IL-10 positive cells was 1.30 in the negative control group, with the highest number observed in treatment group III (K5), and the lowest in the negative control group (KN). The distribution of IL-10 positive cells was normal across all groups ($p > 0.05$). Specifically, the p-values for groups KN, K1, K2, K3, K4, and K5 were 0.585, 0.699, 0.777, 0.807, 0.99, and 0.439, respectively. Because the data were normally distributed and homogenous ($p = 0.357$, $p > 0.05$), the ANOVA test was used to compare groups. The results of the study revealed significant variations between all of the groups (ANOVA, $p = 0.000$), which made it necessary to conduct further research using the Least Significant Differences (LSD) test to identify particular group variations.

Table 2. A comparison between the treatment and control groups' numbers of interleukin-10 (IL-10) positive cells

Group	KN	K1	K2	K3	K4	K5

K N	-	-	-	-	-	-
K 1	0.020	-	-	-	-	-
K2	0.029	0.029	-	-	-	-
K3	0,000	0,000	0.003	-	-	-
K4	0,000	0,000	0,000	0.021	-	-
K5	0,000	0,000	0,000	0,000	0,000	-

Table 2 illustrates the number of positive Interleukin-10 (IL-10) cells across different treatment groups, with most groups showing significant differences ($p<0.05$). In particular, compared to groups KN ($p=0.02$), K2 ($p=0.029$), K3 ($p=0.0$), K4 ($p=0.0$), and K5 ($p=0.0$), group K1 had a considerably higher number of IL-10 positive cells. Similar to group KN ($p=0.0$), group K1 ($p=0.0$), group K2 ($p=0.0$), group K3 ($p=0.0$), and group K4 ($p=0.0$), the number of IL-10 positive cells in group K5 differed significantly from all other groups.

The number of intracellular Interleukin-10 (IL-10) positive cells in pancreatic tissue after streptozotocin infusion was used to determine if mangosteen peel extract prevents inflammation. When compared to the negative control (KN), the first positive control (K1), and the final positive control (K2), the data showed that there was a substantial increase in the number of IL-10 positive cells in each treatment group that was administered mangosteen peel extract. Mangosteen peel extract (K5) at 600 mg/kg BW/day increased IL-10-positive cells the most, while the lowest was in the untreated group, which received neither mangosteen peel extract nor streptozotocin. IL-10 is an antiinflammatory cytokine the body produces to reduce the inflammatory process and suppress proinflammatory mediators. Additionally, IL-10 is involved in immunoregulation and the inhibition of proinflammatory cytokine synthesis, and it directly influences the development of anti-inflammatory cells. Research has shown that mononuclear cells (MNCs) and mesenchymal stem cells (MSCs) have immunoregulatory functions and promote tissue repair by secreting IL-10. Several studies use IL-10 as a marker of the relationship between inflammatory processes and diabetes mellitus. In hyperglycemia, there will be changes in the cytokine Il-10, which can increase pancreatic tissue. This research is in line with other research, which shows that the administration of streptozotocin increases free radicals in the body and triggers an inflammatory process in the creative tissue. However, IL-10 can suppress the increase in proinflammatory cytokines so that the inflammatory process and cell damage can be reduced. The research results show that administering mangosteen peel extract can increase the number of IL-10-positive cells. This was proven by an increase in the number of IL-10 positive cells in the mangosteen peel extract treatment groups (K3), (K4), and (K5). Apart from that, the treatment groups also resulted in differences in the number of IL-10-positive cells. The third dose of 600 mg/kg BW/day (K5) mangosteen peel extract treatment group showed a more significant number of IL-10-positive cells compared to the other treatment groups. This difference is caused by different doses of mangosteen peel extract, which will affect Il-10 in pancreatic tissue. This is because the high antioxidant content will neutralize more free radicals, thereby reducing cell inflammatory processes. In conclusion, administering mangosteen peel extract can help prevent inflammation and mitigate the inflammatory process and cell damage in pancreatic tissue. The increase in anti-inflammatory IL-10-positive cells shows this. Extreme free radicals increase IL-10 production, which can trigger the production of proinflammatory cytokines. Consequently, in cases where free radicals cause inflammation, anti-inflammatory agents, such as those derived from mangosteen peel, are essential for preventing this response.

4. Conclusion and future scope

The research findings indicate that varying doses of mangosteen peel extract have an impact on increasing the number of IL-10 positive cells, with 600 mg/kg BW/day being the most effective dose. Additional research should be conducted to investigate the factors influencing the occurrence of hyperglycemia in humans, particularly by investigating biomolecular aspects.

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Conflict of interest

There is no possible conflict of interest, according to the author

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