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Therapeutic potential of basil (*Ocimum basilicum* L.) aqueous extract: Impact on glycemia and oxidative stress in normoglycemic and diabetic rats

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The prevention of nutrition-related diseases holds paramount significance for human well-being. Sweet basil (*Ocimum basilicum* L., Lamiaceae) emerges as a noteworthy candidate due to its robust antioxidant properties attributed to a high concentration of phenolic and flavonoid compounds, promising potential health benefits for humans. Notably, bile acids and their derivatives exert influence on the metabolic effects of both conventional and herbal drugs. The aim of this study was to examine the effects of administering an aqueous basil extract for seven days on glycemia and *in vivo* antioxidant activity in both healthy and diabetic animals, either alone or in combination with a bile acid derivative. The experimental design involved normoglycemic and diabetic Wistar rats subjected to a seven-day regimen of saline and basil water extract. Hyperglycemia was induced using alloxan. Following the treatment period, animals were euthanized by cardiopuncture, and serum analyses were conducted to assess fasting blood glucose levels and biochemical parameters. Additional assessments included oral glucose tolerance tests and antioxidative stress enzyme assays. The findings showed a significant hypoglycemic effect of the aqueous basil extract in both normoglycemic and diabetic animals. The extract also decreased lipid peroxidation and increased the activity of antioxidant enzymes. Basil extract treatment displayed protective effects on glycemia in both normoglycemic and diabetic animals, indicating considerable antioxidant potential. These effects were evident through increased antioxidant enzyme activity and decreased lipid peroxidation, affirming the beneficial impact of aqueous basil extract on health parameters.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder resulting from pancreatic insulin production dysfunction or tissue resistance to insulin, resulting in elevated blood glucose levels or increased glycosylated hemoglobin A1C. Prolonged periods of high blood sugar levels and microvascular complications associated with DM can result in severe coronary and carotid artery diseases, as well as macrovascular complications affecting the kidneys, eyes, and nerves. Globally, diabetes is responsible for approximately 5.2 million deaths, with an estimated 629 million individuals projected to be affected by 2045. Furthermore, there is a clear correlation between obesity and the onset of diabetes, with obesity serving as a contributing factor to its development. The rapid increase in diabetes incidence can be attributed to various factors, including heightened consumption of processed foods, an aging population, socioeconomic development, and decreased physical activity levels. Due to the initially mild symptoms of type 2 DM, approximately 50% of individuals affected by the condition remain unaware of their diagnosis, leading to unnoticed symptoms and the onset of diabetic complications long before a formal diagnosis is made (Glovaci et al. 2019). Elderly individuals and those with various comorbidities may have reduced life expectancy, limiting their ability to benefit from long-term diabetes management, which includes a decreased risk of vascular complications. Metformin, a medication with a lengthy history of use in type 2 diabetes mellitus (DM), is widely recognized for its well-tolerated nature and is commonly prescribed as initial therapy for effective glycemic control. However, metformin use is contraindicated in cases of severe renal failure and should be avoided in individuals with compromised hepatic function or congestive heart failure (Strain et al. 2021). Changes in lifestyle, exercise habits and especially in the diet, can significantly improve metabolic control, avoid or delay pharmacotherapy and offer wide benefits for patients such as decrease the severity of complications (Taylor et al. 2021). Hence, the latest research is focused on exploring alternative methods to regulate blood sugar levels and mitigate diabetes complications, aiming to enhance diabetes pharmacotherapy.

Currently, issues concerning the management and regulation of blood glucose levels are widespread, primarily not due to a shortage of glucoregulatory medications, but rather due to challenges associated with their utilization. Over the past few decades, numerous studies have validated the effectiveness of plant extracts in addressing prediabetic conditions and diabetes mellitus in humans (Furman et al. 2020). Multiple studies conducted worldwide have revealed the substantial role of oxidative stress in the onset and progression of diabetes mellitus and its complications. Potent antioxidant compounds, like polyphenols present in plants, have emerged as promising agents for the prevention and treatment of this escalating public health issue (Burgos-Morón et al. 2019; Stagos 2019).

In Turkey, *Ocimum basilicum* is traditionally used to prevent and treat diabetes and cardiovascular disorders; in Bulgaria, it is employed for alleviating aches and pains and in Spain, it is used as a sedative (Purushothaman et al. 2018). In Indian traditional medicine, basil is used as an adjuvant therapy for stress, asthma and diabetes. Several studies have demonstrated that basil extract exhibits both hypoglycemic and antihyperglycemic effects on blood glucose levels (El-Beshbishy and Bahashwan 2012; Zeggwagh et al. 2007). The antihyperglycemic effect of aqueous basil extract is attributed to its inhibition of α-amylase and α-glucosidase activity. This action slows

down glucose absorption in the intestinal tract, thereby preventing postprandial hyperglycemia (El-Beshbishy and Bahashwan 2012). Basil acts similarly to insulin in adipose tissue cells, ie increases the transport of glucose from the blood to adipocytes. Thus, basil preparations have a similar mechanism of antihyperglycemic effect as biguanide and thiazolidinedione derivatives (Broadhurst et al. 2000). Bile acids, classified as steroid molecules, assume a crucial role in overseeing glucose metabolism and upholding energy equilibrium (Hou et al. 2023). The precise mechanisms through which bile acids elicit their glucose-lowering effects remains incompletely elucidated. Information gleaned from a range of studies, spanning both laboratory experiments and clinical trials in animals and humans, suggests a variety of mechanisms. These mechanisms include enhancing the release of the incretin hormone glucagon-like peptide-1 (GLP-1) in response to glucose stimulation and activating the nuclear farnesoid X receptor, which plays a role in lipid and glucose metabolism (Mazidi et al. 2017). Bile acids, with a particular focus on the extensively researched 12-monoketocholic acid (MKC), exhibit hypoglycemic and antihyperglycemic effects. They also hinder the transcription of genes necessary for producing the enzyme phosphoenolpyruvate carboxykinase, a critical component in hepatic gluconeogenesis (Teofilovic et al. 2021). Considering that both basil and bile acids have documented antidiabetic activities, the effects of their concomitant administration are worthy of attention. The aim of this study was to evaluate how basil extract, alone or combined with bile acid, affects glycoregulation and antioxidant activity. This research adds to the current knowledge about the beneficial properties of basil and investigates its potential as a complementary medicinal treatment for individuals with diabetes.

2. Investigations and results

2.1. Chemical characterization of basil extract

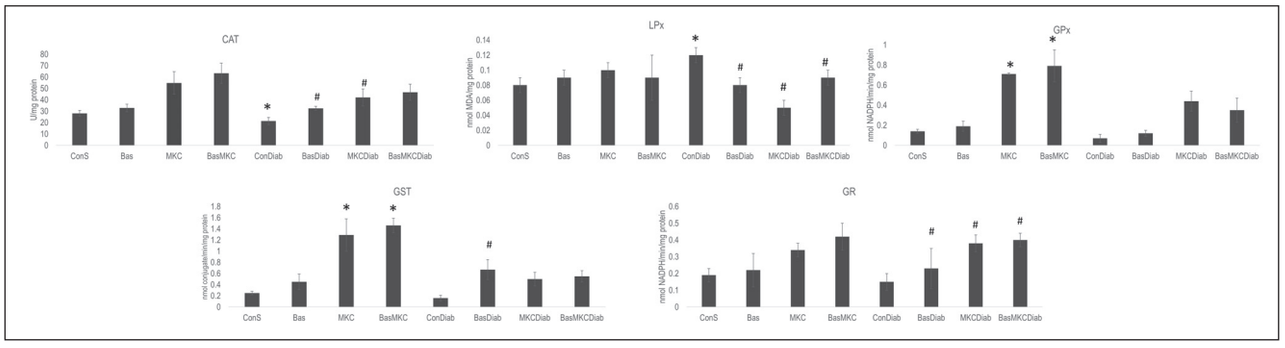
Total yield was 31.22 ± 0.95 g per 100g of dry extract. Total phenolic content was 57.28 ± 1.08 mg galic acid per gram of dry extract and flavonoid content was 4.5 ± 0.18 mg quercetin per gram of dry extract. IC_{50} value for DPPH radical was 0.33 ± 0.05 µg/ml, 11.44 ± 1.02 µg/ml for OH radical, 2.15 ± 0.18 µg/ml for H_2O_2 and 47.00 ± 3.48 µg/ml for lipid peroxidation. Basil extract predominantly contains quercetin (0.1651 mg/g of dry extract). In addition to quercetin, the extract of *O. basilicum* also contained naringenin (0.0706 mg/g of dry extract) and rutin (0.0106 mg/g of dry extract). Phenolic acids were also present: chlorogenic acid (0.0571 mg/g of dry extract), rosmarinic acid (0.0569 mg/g of dry extract), cinnamic acid (0.0055 mg/g of dry extract), p-hydroxybenzoic acid (0.0281 mg/g of dry extract), caffeic acid (0.0574 mg/g of dry extract), ferulic acid (0.0429 mg/g of dry extract) and vanilic acid (0.0518 mg/g of dry extract).

2.2. In vivo antioxidant activity of basil extract

The impact of basil extract on the antioxidant capacity in liver cells was determined by measuring the intensity of lipid peroxidation and concentrations of the antioxidant enzymes in the homogenized liver tissue of rats exposed to alloxan oxidative damage (Fig. 1).

2.3. Antidiabetic activity

Following random assignment to control and experimental groups, body weight and fasting blood glucose levels were assessed both before and after treatment. The alterations in body weight and fasting blood glucose levels are summarized in Table 1.



*p<0.05 compared to ConS, #p<0.05 compared to ConDiab,

Fig. 1: Values of oxidative stress parameters (mean ± SD, n=6)

Table 1: Changes in body weight and glycemic control (%, mean ± SD, n=6)

Group	%ch. BW [%]	BGLstart [mmol/l]	BGLend [mmol/l]	%ch. BGL [%]
ConS	11.4%±0.9%	4.8±0.4	5.4±0.72	11.9%±0.9%
Bas	10.7%±0.7%	5.42±0.5	4.9±0.48*	-9.6%±-0.9%
MKC	23.2%±2.9%	6.2±0.3	5.9±0.2	-4.2%±-0.2%
BasMKC	22.4%±1.7%	6.7±0.8	6.02±0.3*	-10.4%±-1.2%
ConDiab	4.6%±0.3%	25.1±6.7	17.9±11.2	-28.5%±-7.6%
BasDiab	1.9%±0.2%	26±5.6	15.4±4.7*	-40.7%±-8.7%
MKCDiab	0.9%±0.1%	30.7±0.7	19.5±8.6*	-36.4%±-0.8%
BasMKCDiab	1.5%±0.1%	30.7±3.7	5.5±1.04*,#	-82.1%±-9.9%

BW– body weight; BGL – blood glucose level, %ch– percentage change; ConS – saline, Bas – basil extract, MKC – monoketocholic acid, BasMKC – basil and monoketocholic acid, ConDiab – aloxane, BasDiab – alloxan + basil, MKCDiab – alloxan + monoketocholic acid, BasMKCDiab – aloxane + basil + monoketocholic acid; *p <0.01 compared to start values, #p <0.01 in relation to ConDiab, BasDiab and MKCDiab

2.4. Renal and liver function

Serum biochemical parameters (indicators of liver and renal function) are important for diagnosing and assessing safety profile. Hepatosomatic index, serum urea, creatinine and uric acid concentrations were measured as indicators of safety of studied substances

in the present study. In animals treated with alloxan, there was a change in the ratio of liver mass to body mass (Table 2).

No microscopic changes of the liver and kidneys that would be noticeable on light microscopy were found in individuals from all 8 analyzed groups (Fig. 4).

Table 2: Hepatosomatic index, concentration of direct bilirubin, AST, ALT, uric acid, urea and creatinine (mean ± SE; n = 6)

	HSI (%)	Direct bilirubin (µmol/L)	AST (U/I)	ALT (U/I)	Uric acid (µmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
ConS	26.78±2.72	0.35±0.06	138.00±5.00	25.50±1.147	64.33±8.24	9.07±0.62	49.17±4.30
Bas	26.95±1.54	0.32±0.07	136.83±8.36	22.00±2.733	66.50±9.17	9.30±0.66	56.33±4.19
MKC	26.68±1.38	0.33±0.06	135.17±13.81	24.83±2.892	65.00±6.30	5.46±0.29*	35.00±0.36
BasMKC	27.69±2.35	0.32±0.05	126.67±14.52	25.33±5.731	71.83±8.61	5.13±0.24*	33.17±0.83
ConDiab	29.2±3.27*	0.65±0.04**	170.67±12.78**	45.50±2.43**	99.33±9.60**	10.03±0.57**	60.67±4.58**
BasDiab	25.18±3.1	0.63±0.08	148.33±11.83	24.83±3.88$^{\#}$	81.83±10.19	9.85±0.54**	56.17±4.21
MKCDiab	24.66±1.71	0.42±0.08	146.50±2.66$^{\#}$	30.17±4.58	78.33±4.25	11.98±1.80**	38.00±1.71$^{\#}$
BasMKCDiab	24.16±1.35	0.27±0.03$^{\#}$	131.67±13.15$^{\#}$	34.17±4.14	69.00±3.31$^{\#}$	8.82±0.58**	32.50±0.76$^{\#}$

*p <0.01compared to all other diabetes groups, **p <0.01 compared to ConS, $^{\#}$p <0.01 compared to ConDiab

2.5. Histological and morphometric analysis of pancreatic and renal tissues

Immunohistochemical analysis of pancreatic tissue from analyzed groups confirmed preserved cytoarchitectonic arrangements of endocrine and exocrine compartments (Fig. 2).

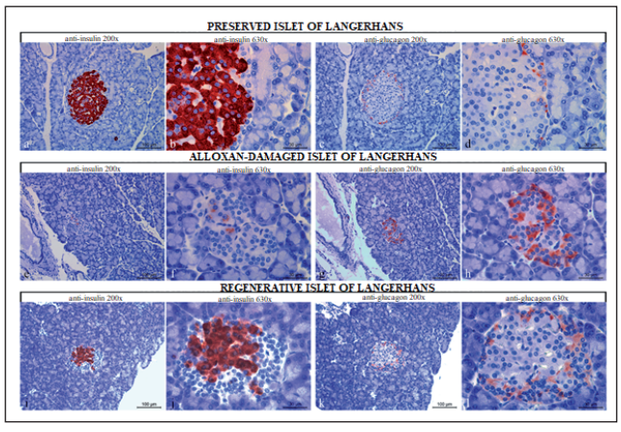


Fig. 2: Histological characteristics of Langerhans islets of pancreatic tissue, immunohistochemically stained for detection of insulin and glucagon.

The analysis of the surface density of the endocrine and exocrine part of the pancreatic tissue in animals treated with alloxan revealed statistically significant lower density of endocrine pancreas when comparing with control group (Cons) (Fig. 3 a, b).

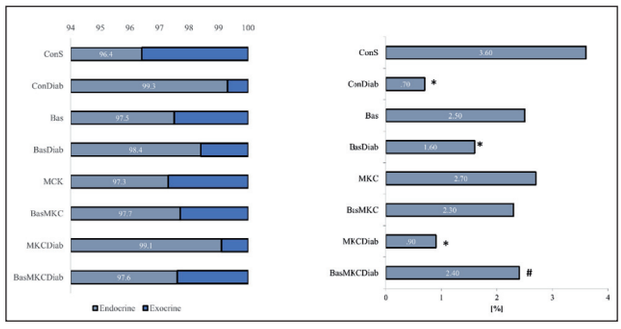


Fig. 3: Morphometric analysis of surface density of endocrine and exocrine part of pancreatic tissue, b: Mean values for the percentage of surface density of the endocrine pancreas; *p<0.05 compared to ConS, $^{\#}$p<0.05 compared to ConDiab.

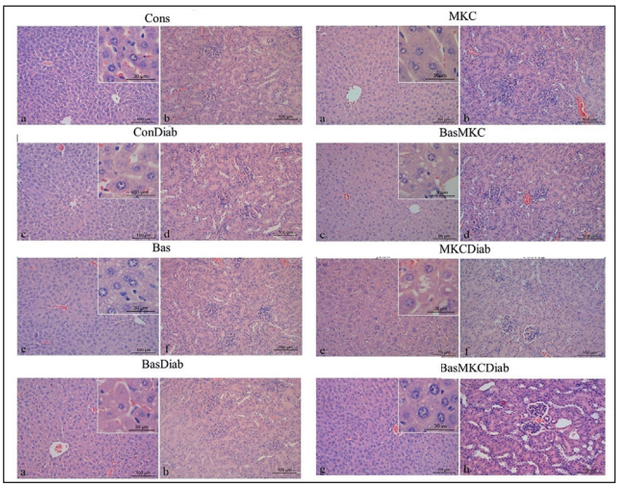


Fig. 4: Histological characteristics of liver and kidney tissue, hematoxylin and eosin staining, scale = 100µm

3. Discussion

Studies have shown that quercetin modulates the function of various antioxidant enzymes, including glutathione reductase, glutathione peroxidase, glutathione-S-transferase, ascorbate peroxidase, superoxide dismutase, and peroxidase enzymes. The effectiveness of quercetin appears to be dependent on dosage, with an optimal dose being advantageous for specific plant species (Singh et al. 2021). Phenolic acids play diverse roles in plants, serving as structural elements of the cell wall, facilitating nutrient absorption, promoting seed germination in challenging environmental conditions, functioning as signaling molecules in both local and systemic plant defense mechanisms, and influencing the composition of beneficial microbial communities in the rhizosphere soil (Al Jitan et al. 2018).

The collected findings demonstrate that the basil's aqueous extract decreased the level of lipid peroxidation induced by alloxan, which aligns with previous studies that have verified the antioxidant properties of examined extract (Fig. 1) (Kaurinovic et al. 2011). Erythrocytes predominantly contain the enzyme catalase which is an essential enzyme in preventing against oxidative damage. Catalase promotes the dismutation of hydrogen peroxide into harmless compounds, protecting membrane proteins and lipids from injury triggered by peroxyl radicals (Matsumoto et al. 2020). The administration of basil solution resulted in an elevated catalase reaction in a group of diabetic rodents which received MKC, as well as in a group receiving both, a basil and MKC. The obtained findings

demonstrate the significant ability of basil and MKC in providing protection against harmful oxygen species. The study conducted by Dasgupta et al. found that the application of an alcoholic-aqueous extract of basil improved the activity of catalase and protected against the cancer-causing and oxidative effects of benzo(a) pyrene and 7,12-dimethyl benzo(a)anthracene in Swiss albino mice (Dasgupta et al. 2004). As expected, according to available data from the literature, the use of *O. basilicum* extract has led to an increase in the activity and enzymes GPx, GR and GST, i.e. the protective effect of basil is manifest. The increased levels of these enzymes can be interpreted as a compensatory reaction of cells to oxidative harm (Al-Snafi 2021). Our study found that the groups treated with basil extract showed a significant increase in GR enzyme activity compared to the group given a sodium chloride solution ($p<0.05$). The observed increase suggests that basil extract has a preventive effect against oxidative damage generated by alloxan. Following a seven-day administration of basil extract to diabetic rats, there was a significant rise in GST enzyme levels compared to the group treated with saline ($p<0.05$). Furthermore, the antioxidant properties of basil were evident through the observation of alterations in GST activity in healthy animals. The activity of GST showed a statistically significant increase ($p<0.05$) after treatment with MKC separately or together with water-based basil extract. Alloxan application resulted in a reduction of GPx levels, with a reduction in activity reported in rats treated with a physiological solution. The oral administration of MKC, separately and in combined with basil extract, resulted in a significant rise in GPx activity ($p<0.05$). These findings confirm the protective role of both treatments in animals exposed to oxidative stress.

Treatment of experimental animals with pro-oxidative substance, alloxan, lead to a significant change in body weight (Sabo et al. 2010). In the alloxan treated groups, the weight gain was significantly lower compared to non-diabetic animals (Table 1). The results obtained are in line with existing literature, indicating that the administration of alloxan resulted in disrupted metabolism and impaired weight gain, due to increased breakdown of muscle and fat for energy in the absence of effective glucose utilization (Vidović et al. 2012; Arfa and Rashed 2008). Following seven days of treatment, normoglycemic animals administered with aqueous basil extract exhibited a notable decrease in blood glucose levels compared to those treated with saline, signifying a statistically significant difference. Previous research suggests that aqueous basil extract can swiftly lower blood glucose levels in normoglycemic rats without inducing long-lasting effects on glucose regulation. This transient reduction is attributed to the intact regulatory mechanisms in normoglycemic animals, notably decreased glucose consumption, and heightened gluconeogenesis stimulation in the liver (Gerich 1988). In rats with alloxan-induced diabetes, administration of aqueous basil extract over seven days resulted in a notable reduction in blood glucose concentration compared to pre-treatment levels, underscoring the antihyperglycemic and hypoglycemic properties of basil. These findings are consistent with previous research, corroborating the efficacy of basil extract in managing blood glucose levels in diabetic conditions (Zeggwagh et al. 2007). Scholarly sources suggest that the anti-hyperglycemic effects of basil extract stem from its ability to impede the activities of α-glucosidase and α-amylase enzymes. By doing so, basil extract retards the absorption of glucose in the gastrointestinal tract, thus mitigating the onset of postprandial hyperglycemia (El-Beshbishy and Bahashwan 2012). Moreover, basil demonstrates effects on insulin-target cells within the target tissues, enhancing the uptake of glucose from the bloodstream. This action mirrors the mechanisms observed with thiazolidinediones and biguanides (Broadhurst et al. 2000). Furthermore, administering basil in combination with monoketocholic acid to diabetic animals resulted in a notable reduction in blood glucose levels compared to diabetic animals treated with saline, basil and MKC alone. One of the explanations for observed differences include pronounced antioxidative activity of basil, being rich in polyphenols and flavonoids, as we have confirmed *in vitro*. Antioxidant properties of basil can help protect cells from oxidative stress, associated with alloxane administration and improve glucose control (Teofilovic et al. 2021, 2021c). Bile acids have been observed to suppress the expression of genes involved in the production of phosphoenolpyruvate carboxykinase, a key enzyme in hepatic gluconeogenesis (De Fabiani et al. 2004). Therefore, *in vivo* antioxidant activity of basil extract, alone or in combination with MKC was determined in liver tissue homogenates.

In this study, the ratio of body weight and liver mass at the end of treatment was monitored in animal groups, where a significant difference in the ratio of the aforementioned parameters was observed in the saline-treated alloxan group compared to all other alloxan groups (Table 2). This difference indicates possible hepatotoxicity in a group that did not receive basil extract, thus demonstrating the protective action of basil extract (Etuk 2010). One week separately basil extract treatment or together with MKC, demonstrated improvement in the biochemical parameters that represents liver function in diabetic rats. Both, extract and MKC pretreatment effectively avoided an elevation in the levels of direct bilirubin, AST, and ALT ($p<0.05$). ALT and AST are enzymes that facilitate the transfer of the α-amino group from alanine or aspartate to the α-keto group of ketoglutaric acid. This process leads to the generation of oxaloacetic and pyruvic acids, which play an important role in the Krebs cycle. Hepatocytes have a significant amount of these two enzymes, making their serum concentrations a reliable predictor of liver injury (Rašković et al. 2014; Touiss et al. 2021). An administration of basil extracts independently or combined with MKC in the diabetic animals showed decreased serum levels of creatinine, urea, and uric acid in comparison to the group treated with physiological solution ($p<0.05$). This suggests that the kidney function had been preserved by the basil extract treatment. Earlier studies (Renovaldi and Adam 2020; Al-Snafi 2021) have also verified the correlation between kidney and liver functioning through the measurement of creatinine, urea, alanine transaminase, and aspartate transaminase levels in serum.

Insulin staining clearly showed the central positioning of beta cells within the islet of Langerhans (Fig 2 a, b). In contrast, glucagon-producing alpha cells occupied the peripheral parts of the islet (Fig. 2 c, d). This spatial arrangement of the mentioned cells inside the islet is typical for rodents and represents a normal histological finding. There was clear, selective damage to the beta cells of the islet of Langerhans inside the pancreas in all cases. In a significant number of islets, beta cells were absent, reduced to a small number of insulin-positive cells (Fig. 2 e, f). The remaining cells of the islet of Langerhans showed immunohistochemical staining for glucagon (Fig. 2 g, h). Among the groups in which alloxan was used, BasMKCDiab group stands out. Although there is a reduced surface density of the endocrine part of the pancreas, it does not differ significantly from the control group, indicating that basil together with MKC lead to protection against alloxan. In groups BasDiab and BasMKCDiab, a more significant number of regenerative islets were found, what is in accordance with other similar studies (Tandi et al. 2021; Chaudhary et al. 2016). A larger number of beta cells could be observed that filled the central part of the islet (Fig. 2 i, j). A clear sign of the regenerative characteristics of these islets is the existence of a prominent number of marginally placed glucagon-producing alpha cells, which tells us that the use of basil has a positive effect on the alloxan-damaged endocrine part of the pancreas which is in agreement with the results of other recent studies, but unlike ours, they used streptozotocin for the induction of hyperglycemia and basil seeds extract for rat treatment (Fig. 2 k, l) (Parikh and Kothari 2020).

The correct cortical and medullary border could be seen inside the kidney. Inside the cortex, a significant number of preserved glomerulus were surrounded by numerous straight and curved proximal and distal tubules lined with normal cuboidal epithelium. Focally presence of interstitial lymphocyte was noticed, but this was not marked as a pathohistological finding. The renal collecting systems are composed of regular cuboidal epithelium without visible morphological changes. Histological assessment of the liver tissue across all experimental cohorts determined to the greatest extent the preservation of the cytoarchitectonic structure

of the liver lobule with the correct radial arrangement of Remak beds. The isolated application of alloxan did not lead to microscopically visible changes at the level of hepatocytes, namely, they, like the control group of individuals, were characterized by acidophilic homogeneous cytoplasm and a centrally positioned regular nucleus. Damage caused by alloxan administration was noticeable by in increase in biochemical parameters and antioxidant status, but not significant microscopical findings, which can be attributed to the beneficial effects of basil (Almalki 2019). Although studies that have examined the antioxidant capacity of basil differ methodologically, whether in vitro or in vivo, all of them strongly indicate a positive pharmacological effect of this plant (Othman et al. 2021, Kanmaz et al. 2023). Observing only in vivo research, antimutagenic, antiproliferative, cardioprotective, neuroprotective and other useful pharmacological properties of basil-derived preparations are reported in the literature. The mechanism of these actions is primarily explained by the fact that active compounds from basil prevent the development of all harmful consequences of oxidative stress, in different ways and in different tissues (Azizah et al. 2023, Teofilović et al. 2021c).

The combination of basil extract and monocetoholic acid (MKC) demonstrates significant potential in addressing metabolic and oxidative stress-related disorders. This combination exhibited notable hypoglycemic effects in diabetic animal models, showed promise in preventing metabolic syndrome-related complications. Basil and MKC significantly reduced lipid peroxidation caused by alloxan toxicity and enhanced the activity of key antioxidant enzymes, such as glutathione transferase, reductase, peroxidase, and catalase, in animals under oxidative stress. The combination also inhibited the increase in direct bilirubin and serum aminotransferase levels while preserving renal function. Immunohistochemical and morphometric analyses further revealed regenerative effects on pancreatic Langerhans islets in alloxan-induced damage, highlighting the potential of combination of basil and MKC to support pancreatic endocrine regeneration. These findings underscore the synergistic potential of basil extract and MKC as a therapeutic strategy for metabolic and oxidative stress-related conditions.

4. Experimental

4.1. Plant material and preparation of the extract

The stems of basil plant are tufted and branched, usually bare and pleasantly scented with balsam. The leaves are ovate, pointed at the top, and coarsely serrated. The flowers are yellowish-white or reddish, arranged in clusters in the axils, stacked one above the other. It grows from India, South Asia, North Africa to the tropical part of America, and is cultivated. The aerial components of basil were acquired from the Institute for Medicinal Plant Research "Dr. Josif Pancic" in Belgrade (Basilici herba, voucher No: 14910513). To prepare the extract, an herbal infusion method was employed, which entailed pouring 1 g of the herb into 200 ml of hot water, following the recommended guidelines. Subsequently, the extract underwent filtration, evaporation to dryness, and quantification of the extraction yield (dry extract, d.e.).

The yield was measured, and the total phenolic and flavonoid content was assessed through spectrophotometric techniques. The antioxidant capacity was determined using spectrophotometric methods to measure lipid peroxidation intensity and the inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxide, and peroxide radicals (Teofilović et al. 2021a). Chemical characterization of basil extracts was performed by high performance liquid chromatography (HPLC). The components were separated using an HPLC column (Zorbax SB C18, 5µm, 4.6 x 150mm). The HPLC mobile phase was freshly prepared daily and filtered through a 0.45 µm nylon filter. Solvent A consisted of 0.1% acetic acid, while solvent B comprised 0.1% acetic acid in acetonitrile. The mobile phase was delivered in gradient mode using the following solvent proportions: 0-3.25min 10% solvent B; 3.25-8min from 10% to 12% solvent B; 8-15min from 12% to 25%; 15-15.8min 25% to 30% solvent B; 15.8-25min from 30% to 90%; 25-25.4min 100% solvent B (Teofilović et al. 2021b).

4.2. Laboratory animals and experimental procedures

During the study, healthy male Wistar rats weighing between 250 and 300 grams were utilized. These laboratory rats were randomly selected from the Military Technical Institute in Belgrade, Serbia. They were housed under standard laboratory conditions at the Department of Pharmacology, Toxicology, and Clinical Pharmacology of the Medical Faculty in Novi Sad, Serbia. The room maintained a temperature of 23-25°C, with a humidity level of 55% ± 1.5% and a 12-hour light-dark cycle. The rats had unrestricted access to standard pellet food and water. All experimental procedures and animal treatments were conducted following the guidelines of the EU Directive 2010/63/EU on animal welfare and adhered to the Law on Animal Welfare of the Republic of Serbia (OG RS 41/09). The entire experimental protocol was performed in accordance with a protocol approved by the Ethics Commission for the Protection of Laboratory Animal Welfare of the University of Novi Sad (Novi Sad, Serbia; No. 01-90/4-4), and it received further approval from the Ministry of Agriculture, Forestry, and Water Management (Belgrade, Serbia; No. 323-07-00550/2014-05).

The animals were randomly divided into 8 groups, each comprising 6 animals. Among these, four groups underwent no alloxan pretreatment, while the remaining four groups were subjected to alloxan-induced diabetes (AID):

- ConS – saline 1 ml per kg (per os)
- Bas – aqueous extract of basil (200 mg per kg body weight, per os)
- MKC – monoketocholic acid (4 mg per kg, per os)
- BasMKC – aqueous extract of basil (200 mg per kg body weight, per os) + monoketocholic acid (4 mg per kg, per os)
- ConDiab – alloxan induced diabetes + saline (per os)
- BasDiab – alloxan induced diabetes + aqueous extract of basil (200 mg per kg body weight, per os)
- MKCDiab – alloxan induced diabetes + monoketocholic acid (4 mg per kg, per os)
- BasMKCDiab – alloxan induced diabetes + aqueous extract of basil (200 mg per kg body weight, per os) + MKC (4 mg per kg, per os)

Both normoglycemic and diabetic animals received identical treatment regimens, consisting of oral administration of either a physiological solution or basil extract. The doses of basil extract and MKC were based on literature data (Umar et al. 2010, Askari et al. 2019, Stević et al. 2018). The extract suspensions were given for a period of 7 days, and on the last day (2 hours following the final dose), the rats were anesthetized using a urethane solution administered intraperitoneally. Once the animals exhibited loss of the righting reflex, they were euthanized by intracardial puncture to obtain an adequate amount of blood as well as liver, kidney, and pancreas tissues for further investigation. All tissue samples were store at -20 °C until further investigation. All animals were weighed at the beginning of the experiment and then again after the 7-day treatment period.

4.3. Antioxidant enzyme activity

All analyses were conducted using spectrophotometric methods. Liver tissues were homogenized at 4 °C in a TRIS–HCl buffered solution at a ratio of 1:3 (w/v). Parameters related to oxidative stress and lipid peroxidation were assessed in these liver homogenates. Determination of the protein concentration in the rough homogenate and cytosolic fractions of the liver was performed using the Biuret method, which is based on the reaction of the peptide bond with copper ions in an alkaline medium. Using a method based on the reaction of thiobarbituric acid with malondialdehyde from the samples, lipid peroxidation (LPx) was measured spectrophotometrically. Malondialdehyde is an accepted indicator of lipid peroxidation and is used in the evaluation of oxidative stress. The specific activity of catalase (CAT) was determined using a method consisting of spectrophotometric monitoring of the decomposition rate of hydrogen peroxide in the presence of catalase. The determination of the specific activity of glutathione reductase (GR) was based on monitoring the oxidation of NADPH, in a reaction in which the enzyme catalyzes the reduction of oxidized to reduced glutathione. The activity of glutathione peroxidase (GPx) is monitored spectrophotometrically, indirectly, by monitoring the drop in absorbance that originates from NADPH. glutathione S-transferase (GST) activity is determined by measuring the absorbance change in the reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with the thiol group of glutathione (Martić et al. 2022).

4.4. Antidiabetic activity

Freshly prepared alloxan (alloxan monohydrate, Sigma Chemicals Co., St. Louis, MO, USA) solution was administered intraperitoneally at a dose of 130 mg/kg for induction of hyperglycemia. After 48h, blood glucose level was measured in blood sampled from a tail vein, to confirm onset of hyperglicemia, and animals with blood glucose levels above 15 mmol/l were included in diabetic groups in the study (Mikov et al. 2008, Rašković et al. 2008). The last measurement of glucose levels was performed on the last day of the experiments. For glucose level measurements a commercial kit with the Accu-Check Active device (Roche Basel, Switzerland) was used.

4.5. Liver and kidney function tests

Function parameters of liver and kidneys were evaluated using serum samples, which underwent analysis for alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin concentrations, urea, creatinine, and uric acid. The Olympus AU 400 autoanalyzer (Hamburg, Germany) and standard kits supplied by the manufacturer were utilized for determining these parameters.

On the last day of the experiment, the body weight of each animal was documented, after which cardiopuncture was performed. Subsequently, a necropsy was conducted, and liver tissue was collected for further examination. Upon removal of the liver, its total weight was measured. The hepatosomatic index (HSI) for each animal was calculated based on these measurements:

$$HSI\ (\%) = 100^{*} \cdot liver\ weight\ (g) / body\ weight\ (g)$$

4.6. Histopathological analysis

For histological assessment, during necropsy, samples of the pancreas, liver, and kidney were taken and afterwards fixed in Bouin's solution at 4°C for 24 hours. This was followed by dehydration with isopropyl alcohol in increasing concentrations (70%, 80%, 96%, and 100%), and embedment in paraffin, and cutting to 5 µm slices on a rotating microtome (Leica, Germany). Staining of tissue sections was performed with hematoxylin and eosin technique, and immunohistochemical staining (for

pancreas tissue). Pancreas tissue samples, using two serial sections from each animal were immunostained for insulin and glucagon with mouse anti-insulin Ab-6 (diluted 1:200; Lab Vision; Thermo Scientific, Rockford, IL) and rabbit anti–glucagon (diluted 1:100; Lab Vision; Thermo Scientific), respectively. UltraVision LP Detection System HRP Polymer & AEC Chromogen (Lab Vision; Thermo Scientific) was used for visualization. For glucagon staining, antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven at 850W for 20 minutes. All antibodies were applied at room temperature for 30 minutes, and counterstaining was performed using Mayer's hematoxylin. Afterwards, slides were mounted in an aqueous-based medium (Bio-Optica, Italy) and coverslipped. Histological slides were examined using a Leica DMLB 100T microscope (Leica, Germany), and details were captured on a Leica MC190HD microscope camera (Leica, Germany). ImageJ 1.45 measurement software was used for the determination of areal density of the endocrine and exocrine compartments of the pancreas.

4.7. Statistical analysis

Statistical processing of the obtained results was performed using the program IBM SPSS Statistics 20.0. The significance of differences in the percentage of liver and kidney damage and the value of the percentage of endocrine pancreatic surface density was assessed by one – way analysis of variance (ANOVA) with Tukey post hoc analysis. Statistically significant differences were considered values of $p < 0.05$.

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Availability of data and materials: All data in this study are available upon request by contacting the corresponding author.

All experimental procedures and animal treatments were conducted following the guidelines of the EU Directive 2010/63/EU on animal welfare and adhered to the Law on Animal Welfare of the Republic of Serbia (OG RS 41/09). The entire experimental protocol was performed in accordance with a protocol approved by the Ethics Commission for the Protection of Laboratory Animal Welfare of the University of Novi Sad (Novi Sad, Serbia; No. 01-90/4-4), and it received further approval from the Ministry of Agriculture, Forestry, and Water Management (Belgrade, Serbia; No. 323-07-00550/2014-05).

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