

# THE EFFECT OF METHANOLIC LEAF EXTRACT OF MENTHA PIPERITA ON ALLOXAN-INDUCED PANCREATIC DAMAGE ON ADULT MALE WISTAR RATS

## ABSTRACT

Type 1 diabetes mellitus is believed to result from destruction of the insulin-producing  $\beta$ -cells in pancreatic islets, mediated by autoimmune mechanisms and is characterized by hyperglycemia. The present study aims to investigate the effect of phytochemical composition of menthapiperita methanolic extract on the pancreas on alloxan-induced type 1 diabetes, and how it affects blood sugar levels. Twenty-eight adult male wistar rats with body weights ranging between 120g – 200g were taken for the study and equally divided into four groups (Groups A-D of 7 rats per group). Group A served as the positive control group, Group B was induced with mint only, Group C was referred as the negative control group for alloxan induced diabetes, Group D, also made diabetic, would be treated with methanolic mint extract at 400mg/kg for 42 days. A single dose of 150mg/kg of Alloxan monohydrate was used to induce diabetes mellitus in male Wistar rats, after which they were tested and confirmed diabetic after 48 hours. Methanolic extract of menthapiperita was administered once daily on the diabetic rats for 42 days. Glucose levels were checked every two weeks before and after the treatment in order to determine the glycemic profiles. The results presented in this study indicate the presence of type 1 diabetes mellitus, and this study strongly suggests the use of *menthapiperita* as a therapeutic intervention for the treatment of type 1 diabetes mellitus.

**KEYWORDS:** Mentha piperita, methanolic leaf extract, alloxan monohydrate, hyperglycemia, diabetes, pancreas, insulin.

## INTRODUCTION

“Diabetes mellitus (DM) is considered to be a syndrome associated with disorders in the metabolism of carbohydrates, lipids, and proteins caused by the absolute or relative lack of insulin” (Yin *et al.* 2017). “This is a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. Diabetes is caused by inherited and/or acquired deficiency in production of insulin by the pancreas (type 1) or by ineffectiveness of insulin (type 2), is one of the most burdensome and costly chronic disease in the world. According to the International Diabetes Federation, 382 million people are suffering from diabetes and diabetes patients will increase to about 592 million by 2035” (Juarez-Reyes *et al.*, 2009). Diabetes can be caused by a

number of biological factors, of which insulin resistance and deficiency are both related to hyperglycemia and hyperlipidemia. Type 1 diabetes, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin by itself. For people living with diabetes, access to affordable treatment, including insulin, is critical to their survival. There is a globally agreed target to halt the rise in diabetes and obesity by 2025.

“The Pancreas is an accessory glandular organ in the digestive system and endocrine system of vertebrates. In humans, it is located in the abdominal cavity behind the stomach” (Gyr and Beglinger et al., 2002). “It is an endocrine gland producing several hormones including, insulin, glucagon, somatostatin and pancreatic polypeptide which circulate in the blood. The pancreas is also a digestive organ, secreting pancreatic juice containing bicarbonate to neutralize acidity of chyme moving in from the stomach, as well as digestive enzymes that assist digestion and absorption of nutrients in the small intestine” (Henderson et al. 2011). “The pancreatic beta cells are extremely vulnerable to damage caused by reactive oxygen species (ROS). A striking example of beta cell vulnerability is the severe damage by Alloxan. Alloxan and streptozotocin are the most popular diabetogenic agents used for assessing the antidiabetic or hypoglycemic capacity of test compounds. Notably, alloxan is far less expensive and more readily available than streptozotocin. On this ground, one will logically expect a preference for use of Alloxan in experimental diabetes studies” (Macdonald et al., 2013).

“*Menthapiperita* is a flowering plant belonging to the Lamiaceae family, it is a cultivated natural hybrid of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint). The utilization of mint oil in mint-flavoured products, fragrances and pharmaceuticals lead to an upsurge in peppermint production over the past few decades” (Hashimoto *et al.*, 2016). In Nigeria, mint plant has its indigenous names common in native dialect; *ewe minti* in Yoruba, *Na’anaa* in Hausa and “*ahuogwu*” in Igbo. The importance of this study is to increase public awareness of the plant’s medicinal properties, so as to adopt the use of *menthapiperita* as a low-cost, easily accessible and natural remedy for lowering blood glucose levels. According to previous studies, *menthapiperita* lowers blood glucose level and blood pressure as well. But no study has been related to its effect on the morphology of the pancreas.

“Pancreatic damage has been the leading cause of diabetes mellitus, which has posed to be a major health problem and challenge in both developed and developing countries” (Mohapatra *et al.*, 2016). “According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetes worldwide and this is likely to increase to 300 million or more by the year 2025” (King *et al.* 1998; Boyle *et al.*, 2001). It is known that many available drugs for the management of diabetes have adverse effects on the body due to the metabolic stress they impose on the body. This has created a need for more natural, less metabolically demanding remedies for the management of such chronic conditions like diabetes

with high success rate in experimental animals. However, limited literature exist on its use to ameliorate diabetes mellitus, hence the need to carry out this research.

“Studies with *menthapiperita* has demonstrated the presence of a wide variety of bioactive compounds that represent a rich resource in phytochemicals of great interest to treat several pathologies” (Maillard *et al.*, 1996). “Some of the benefic biological effects show that this plant may play an important role as anti-oxidant, antinociceptive, antiinflammatory, antimicrobial, anti-carcinogenic, antiviral, anti-allergic and antitumorogenic, indicating its utility in the prevention or treatment of several diseases” (Nair, 2001). “In the extract of the leaves of *M. piperita* are present mainly flavonoids and phenolic acids and some of the compounds are menthol, menthone caffeic acid, acetaldehyde, amyl alcohol, menthyl esters, limonene, pinene, cardial glycosides, phellandrene, cadinene, pugelone, and dimethyl sulfide. The constituent features include alpha-pinene, sabinene, terpinolene, ocimene, diterpenes, gamma-terpinene, steroids, fenchene, alpha- and beta-thujone, coumarin, citronellol, carotenes, tocopherols, betaine, choline, saponin, tannins, and other components” (Lubbe *et al.*, 2013). “The essential oil of *menthapiperita* contains acetaldehyde, amyl alcohol, menthyl esters, limone, phellandrene, pinene, pugelone, and dimethyl sulfide, alpha-pinene, sabinene, ocimene, gamma-terpinene, terpinolene, alpha- and beta-thujone, citronellol, menthol, menthone, menthofuran, menthyl acetate, isomenthone and other compounds capable of producing the above mentioned effects of this plant” (Djanane *et al.*, 2012).

“These phytochemicals paved the way for significant utilization in the production of pharmaceuticals food and beverage industry. Numerous species of *Mentha* are used as spices and for herbal teas. Generally, every part, for instance, the leaves, stems, and roots of *Mentha*, have been used in tribal and traditional medicine” (Trevisan-Menezes, 2017). “Furthermore, we may say that *menthapiperita* is a promising plant that may offer low-cost alternative strategy for the use in Medicine and in food industry. They also act as antioxidants that delay or prevent the oxidation of inter- or intra-cellular oxidizable substrates from oxidative stress. It has been reported that oxidative stress leads to excessive production of free radicals which are implicated in pathogenesis of diabetes and its complications” (Bayani *et al.*, 2012). “*Mentha* is a medicinal and economically important plant that is regularly used for the treatment of vomiting and nausea, its antiallergic effects, its antifungal and antibacterial effects, its antidiabetic effects, the treatment of obesity, the treatment of gastrointestinal diseases, its anticarcinogenic effects, and pain relief” (Mahsan, *et al.*, 2002). Thus, the WHO study groups has recommended the need for the development and evaluation of a better, safer and affordable pharmacological agents. Recently, anti-diabetic natural products from plants has received great attention from scholars and institutions at large.

“There are also some reports of mint having wound healing and repair properties. In diabetic patients, poor wound healing and diabetic foot syndrome (DFS) continue to be major health problems” (Suranet *et al.*, 2016). “Poor management or neglecting wounds in diabetics can lead to DFS, bad ulcerations and amputations (these patients have high levels of mortality). Moreover, the risk of poor wound healing in diabetic patients increases in parallel with the duration of diabetes and age” (Jain *et al.*, 2016). “Using medicinal plants as a remedy is trusted in many cases, and mint is regarded as one of the important and cheap herbal products. *Mentha piperita* possesses various secondary metabolites which are useful against different disorders. It decreases the glycemia, cholesterol, LDL-c, VLDL-c and triglycerides in the offspring of diabetic rats” (Barbalhoet *et al.*, 2011). “*Mentha* juice may help in the prevention of diabetes and its complications in diabetic rat offspring” (Barbalhoet *et al.*, 2002). “Reduction of blood levels of cholesterol, triglycerides, LDL-c and glucose. *Mentha piperita* may prevent diabetes and its complications” (Mesbahzadehet *et al.*, 2002). “Reduction of blood lipids and exhibits antioxidant activity. Peppermint may help prevent cardiovascular diseases” (Badal and Sharafiet *et al.*, 2001). “Immunomodulatory and anti-inflammatory action in murine model, it exhibits and immunomodulatory and antiparasitic effect in the experimental murine model of schistosomiasis” (Zaiaet *et al.*, 2012).

“*Mentha* exhibits a strong antimicrobial potential, which is why it is considered as one of the most industrially, medicinally, and economically important plant genera. *Mentha* has shown a significant antibacterial resistance against the epidemic bacterium *Chlamydia*” (Valsala, *et al.* 2017). “Additionally, *Mentha* helps fight pneumoniae associated with respiratory disease. A study conducted by Hussain *et al.* reported a strong antibacterial potential of various *Mentha* species. Another study found *Mentha* extracts to have an effective inhibition activity against various strains of bacteria, including *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumoniae*, and *Styphylococcus aureus*” (Nascimento, Rodrigues, Campos, & Costa, 2009). Mimica-Dukić *et al.* isolated “secondary metabolites from *Mentha* and tested them against *Escherichia coli* and *Shigella sonnei*; they showed significant antibacterial activity. Furthermore, using *Candida albicans* and *Trychophyton tonsurans*, studies have shown that *Mentha* extracts have strong antifungal properties”. Another study by López *et al.* reported the “potential of *Mentha* extracts against *Rhizopus stolonifera*”. “Apart from this, various species of *Mentha* have been shown to possess potential antimicrobial activity against resistant pathogens, indicating that metabolites of *Mentha* species are highly active against pathogenic organisms” (Guigueret *et al.*, 2017). “The antimicrobial mechanism of *Mentha* extracts involve the production of antioxidant agents which disrupt the microbial membrane, and subsequently, damage the cellular organelles” (Godghateet *et al.*, 2016). The strong antimicrobial potential of *Mentha* extracts proved it as a highly essential preservative in the food industry. Further studies are required to find which kinds of extracts and which elements are important for the production of health-oriented food. *Mentha* plants contain constituents with cytotoxic properties, and could be used in developing anticancer agents; for example, *M. longifolia*, *M. arvensis*, and *M. × piperita* were

found to possess cytotoxic activity against breast cancer in humans and human laryngeal epidermoid carcinoma. According to Ferreira *et al*, menthol is one of the main components of the essential oil of *M. piperita* that produce anti-cancer activity inducing cell death, either by necrosis or apoptosis (in Caco-2 cell line). The cytotoxicity associated with essential oil has been attributed to various effects such as the production of reactive species, change in fluidity and membrane permeability, tubulin polymerization, imbalance in ion transport, and inhibition of protein function.

## **METHODOLOGY**

### **Study Area**

The study was conducted at the animal research facility of Faculty of Basic Medical Sciences, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Anambra State.

### **Procurement of Alloxan and experimental animals**

Alloxan monohydrate was purchased from a government licensed drug store at Nnewi, Anambra state. Alloxan monohydrate was injected intraperitoneally using a single dose at 150mg/kg body weight after 12-hours fasting state. Twenty-eight (28) adult male Wistar rats weighing between 110-180g were purchased from “the-best” farm in Okofia, Nnewi South L.G.A and acclimatized for two weeks at the Animal house, College of Health Sciences, NnamdiAzikiwe University, Okofia, following the guide for the care and use of laboratory animals (The Guide for the Care and Use Of laboratory Animals, 2011). The rats were housed in stainless steel cages, supplied with fresh water and standard pellet diet *ad libitum*. They were maintained under standard conditions of 12 h light/dark cycle; 25°C; 45%-55% humidity.

### **Collection and Preparation of Methanolic Extract Of Mentha Piperita Leaf**

The fresh and healthy green leaves of *menthapiperita* was collected from a local farm in Jos, Nigeria during April 2023. The leaves were thoroughly washed with clean running water to remove dirt and soil particles. *Mentha piperita* leaves was authenticated by Mr. IrokaFinian, a taxonomist at the Nnamdi Azikiwe University Herbarium, awka, Anambra state. Leaves are separated and air dried under shade, the dried plant material was grinded by a heavy duty blender, The powder was extracted with methanol using the Maceration method, The coarse powdered mint was soaked in 2 liters of methanol and left for about 48 hours, The extract was filtered with a porcelain cloth and its chaff discarded. The filtrate was concentrated in a rotary evaporator (Digital) TT-52 (Techmel&Techmel USA), stirred and boiled at 65°C, the equivalent boiling point with the solvent. The concentrated extract was oven dried with a laboratory oven TT-9023A at 40°C for 3 days until it was in jelly-like form. It was freeze dried for 48 h. The freeze dried extracts was stored at -20°C until use.

### **Determination of Median Lethal Dose (LD<sub>50</sub>)**

The Median Lethal Dose (LD<sub>50</sub>) of the methanolic leaf extract of *menthapiperita* was determined using a New Method for Determining Acute Toxicity in Animal Models as described by Enevide et al; (2013). This method was carried out in three stages with the outcome of each stage determining whether to terminate or move on to the next stage. Eight days of full acclimation of experimental rats. All twelve (12) experimental rats used, weighed about 120-180g and their mean body weight calculated. A stock solution of 1g of methanolic extract of mint in 10ml of distilled solution was prepared.

### **Phytochemical Analysis**

The phytochemicals present in the leaves of *menthapiperita* sample extracted were studied. The organic solvent of methanol was used for the preparation of extract for the study. The methanolic extraction of peppermint showed the presence of phenols and tannins, flavonoids, carbohydrates, glycosides and alkaloids. The extract was analyzed for the qualitative and quantitative phytochemical analysis.

### **Experimental Design**

A total of twenty-eight (28) apparently healthy adult male Wistar rats weighing (110-170g) were randomly selected and distributed into four groups of seven animals each (A, B, C, D), according to their weight. They were acclimatized for 14 days before administration.

The entire group were fed with vital feed and water *ad-libitum* on daily basis and weighed every two weeks. A single freshly prepared 150mg/kg dose of Alloxan was used to induce pancreatic damage on groups C and D. Type 1 diabetes mellitus was confirmed by the presence of high fasting plasma glucose level (above 212.4mg/dl) which was determined 48 hours after inducing alloxan. Its effect on fasting blood glucose level was noted. Methanolic extract of *menthapiperita* was then administered once daily on diabetic rats for 42 days. Blood samples were collected before and after the treatment in order to determine the glycemic profiles. Group A – served as the control group; and were fed only with vital finisher feed *ad libitum* throughout the duration of the experiment; Group B – Mint group only; non-diabetic rats administered with a dose of 400mg methanolic extract of *menthapiperita*; Group C – Alloxan only; received 150mg/kg of alloxan monohydrate and served as the negative control group for alloxan-induced diabetic rats without treatment; Group D – Alloxan and mint; received 150mg/kg of alloxan monohydrate and served as the alloxan-induced diabetic rats administered with 400mg/kg methanolic extract of *menthapiperita*.

All extract administration was given orally and lasted for 42 days. Blood samples were collected from the tail artery of the rats and their fasting blood glucose were determined after 12 hours fast

by using ACCU-CHEK active blood glucose monitoring system and accu-check active test strips on bi-weekly basis and their results expressed in mg/dl.

### **Blood Analysis on Insulin**

Hormonal assay test was performed to give an indication of metabolic processes and conditions or 'insulin imbalance'. Insulin hormone test was performed on three samples of blood from all four groups to measure the levels of serum insulin in blood. This is to confirm hormonal abnormalities especially after treatment. Insulin Hormone Test was carried out using Accu-Bind ELISA Microwells method, Insulin Test System Product Code: 2425-300. This test was performed at Onamech Laboratory, Umudim, Nnewi.

### **Statistical Analysis**

Research objectives and hypothesis of the study were considered before analyzing data. Results obtained were expressed in mean  $\pm$  standard error mean where applicable. The data were analyzed using paired samples t-test, analysis of variance (ANOVA) and post Hoc LSD multiple comparison of groups. Significant differences were obtained among means using Duncan's Multiple Range Test at  $p < 0.05$ .

## **RESULT AND DISCUSSION**

### **Observations**

At the beginning of the experiment, all animals were apparently healthy and agile. Adult male Wistar rat is characterized by wide head, long ears and a tail length with pinkish eyes, smooth white hairs, dark brown stool etc. Animals showed signs of heavy breathing, Weakness and fatigue, Loss of appetite and frequent urination, little or no movement, High mortality rate.

N/B: After the second week of inducing pancreatic damage with Alloxan, the animals feeding habits and urine outputs returned to normal.

The antidiabetic, hypoglycemic and therapeutic effects of methanolic leaf extract of *Mentha piperita* was tested and confirmed against the diabetogenic effect of alloxan monohydrate on the blood sugar levels, insulin and the pancreas. Exposure of Groups C and D to alloxan monohydrate caused severe damage to the endocrine pancreas with a highly significant decrease in the insulin levels. The healing effect of *menthapiperita* was evaluated on the cells of the pancreas (group D) which showed a moderate regeneration after cellular necrosis of the pancreas. Although all *in vitro* experiments hold limitations with regard to possible *in vivo* efficacy, the results of this study deserve attention with regard to antioxidative and possible anti-diabetic therapy that form a basis for future research. Even though essential oils might not be ideal for the treatment of human cancers, the oil tested certainly deserves some further investigation.

*Mentha piperita* leaf contain naturally dynamic synthetic substances for example Ammodendrine, Tannin, spartein, proanthocyanidin, steroids, flavononesetc which were found in high quantity in fresh samples of methanolic leaf extract while moderately present in dried sample. This may be due to the moisture content in fresh sample which yielded concentration of constituents to be higher, this finding is in line with Erukainure *et al.* 2017, Najafi 2013, Heleno *et al.* 2013. Diabetes mellitus is a complicated disorder that results from impairment of insulin action or secretion seen in chronic hyperglycemia and long term severe complications (Ifedi *et al.*, 2021).

The study reports the median lethal dose of methanolic extract used showed zero mortality with toxicity above 5000mg/kg. The study findings showed a significant ( $p < 0.05$ ) increase in body weight in non-diabetic groups A and B, while groups C and D had a non-significant increase in bodyweight. The report of Olivia (2020) revealed a non-significant decrease in the bodyweight following alloxan-induced diabetic treatment, this contradicts the study report. However, the study findings disagree with the report of Ewenighiet *al.*, (2015) which reveals significant weight loss following alloxan toxicity. The report of Obia (2016) showed a significant lower bodyweight following alloxan treatment in diabetic model, which contradicts study findings. Mechanism of action for *menthapiperita* shows a non-significant increase in bodyweight of infected animals.

At Week 0 (before extract administration); study showed a significant increase ( $p < 0.05$ ) on the effect of *menthapiperita* on blood glucose level on Group D, group B (mint only) showed a non-significant ( $p > 0.05$ ) decrease in blood sugar levels when compared with group A (Control). Group C (Alloxan only) showed a non-significant decrease in glycemc profile in comparism with group A (control). At Weeks 2, 4 and 6 of *menthapiperita* leaf extract administration; study reports groups C (alloxan only) and D (alloxan and mint) showed a non-significant increase on the effect of *menthapiperita* on blood sugar level in comparism with group A. Group B (mint only) showed a non-significant decrease in blood sugar level when compared with group A. Group A (control) was used in comparison to groups B, C, D to show the effects of methanolic leaf extract on *menthapiperita* on blood glucose levels. Group D (alloxan and mint) showed an initial significant increase in blood sugar level which later showed a non-significant decrease after 42 days of administration 400mg/kg methanolic leaf extract of *menthapiperita*. This study has similar report with Aroda and Ratner (2018) revealing a significant decreased blood glucose level following metformin administration.

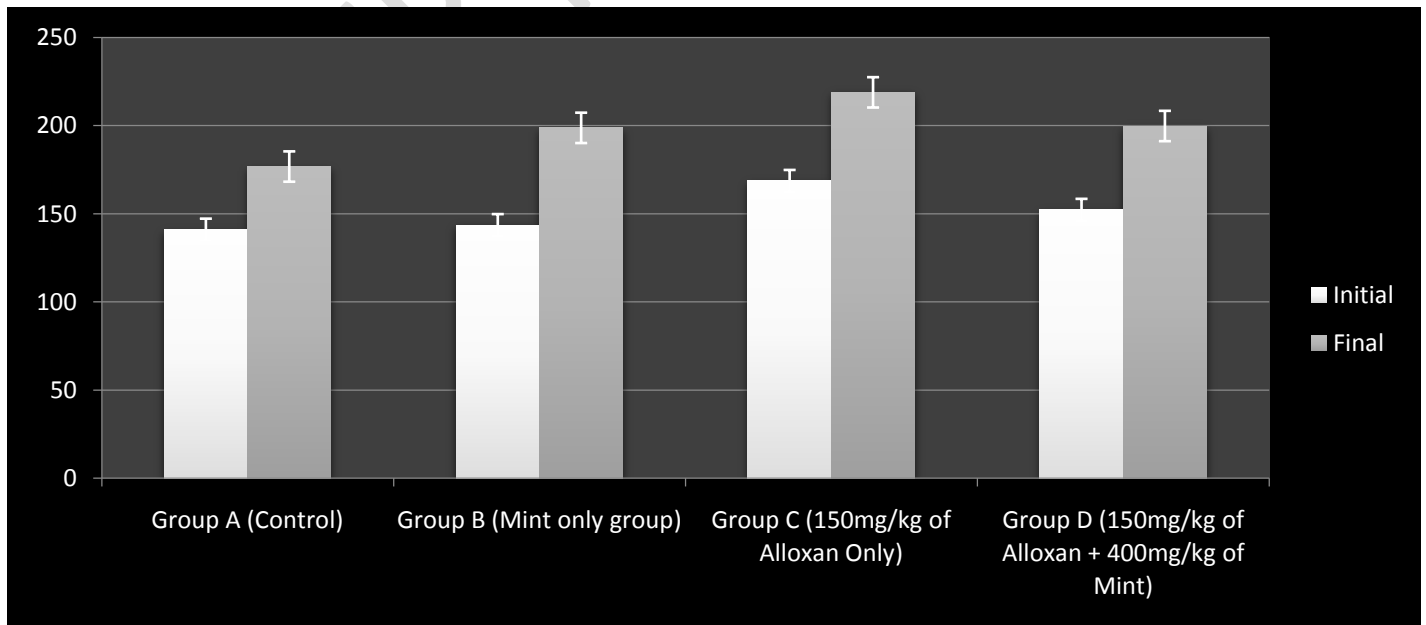
The study on insulin levels revealed significant decrease between pairs of diabetic groups C (alloxan only) and D (alloxan and mint) in comparism with group A (Control), group B showed a non-significant decrease in insulin levels in comparism with group A. The physiology behind the decrease could be attributed to the presence of ROS generation leading to insulin insensitivity (Oliveira *et al.*, 2020). These findings indicates that groups C and D are more susceptible to diabetes mellitus than A and B. Diabetes mellitus results from the impairment of insulin action or

secretion seen in chronic hyperglycemia (Ifediet *al.*, 2021). Insulin levels of groups A (control) and B (mint only) were significantly higher than that of C and D. This results confirms pancreatic damage of beta cells in groups C and D. After treatment, the increase in insulin levels of group D (alloxan and mint) when compared with group C (alloxan only) revealed the presence of flavonones in the extract has the potency to alleviate damage caused by alloxan. This study agrees with Abo-Youssef and Messiha (2013), Oluwaleet *al.*, (2018), revealing a significant reduction in insulin level following alloxan toxicity.

**Table 1: Effect of methanolic leaf extract of *menthapiperita* on the weight of alloxan-induced pancreatic damage on adult male wistar rats.**

Groups	Weight(g)	Mean±SEM	t-Value	Mean Difference	p-Value
Group A	Initial	141.08±3.74	-5.022	-35.7250	.015*
	Final	176.8±10.12			
Group B	Initial	143.58±2.95	-3.534	-55.1750	.039*
	Final	198.75±12.79			
Group C	Initial	168.63±8.89	-3.759	-50.2667	.064
	Final	218.90±6.52			
Group D	Initial	152.53±0.58	-1.679	-47.2667	.235
	Final	199.80±27.79			

**Table 1;** Data was analyzed using a paired sample t-test and results were considered significant at  $p < 0.05$ . Results showed a significant ( $p < 0.05$ ) increase in the bodyweight in groups A and B (non-diabetic groups); Groups C and D (Diabetic groups) showed a non-significant decrease ( $p > 0.05$ ) in the bodyweight.

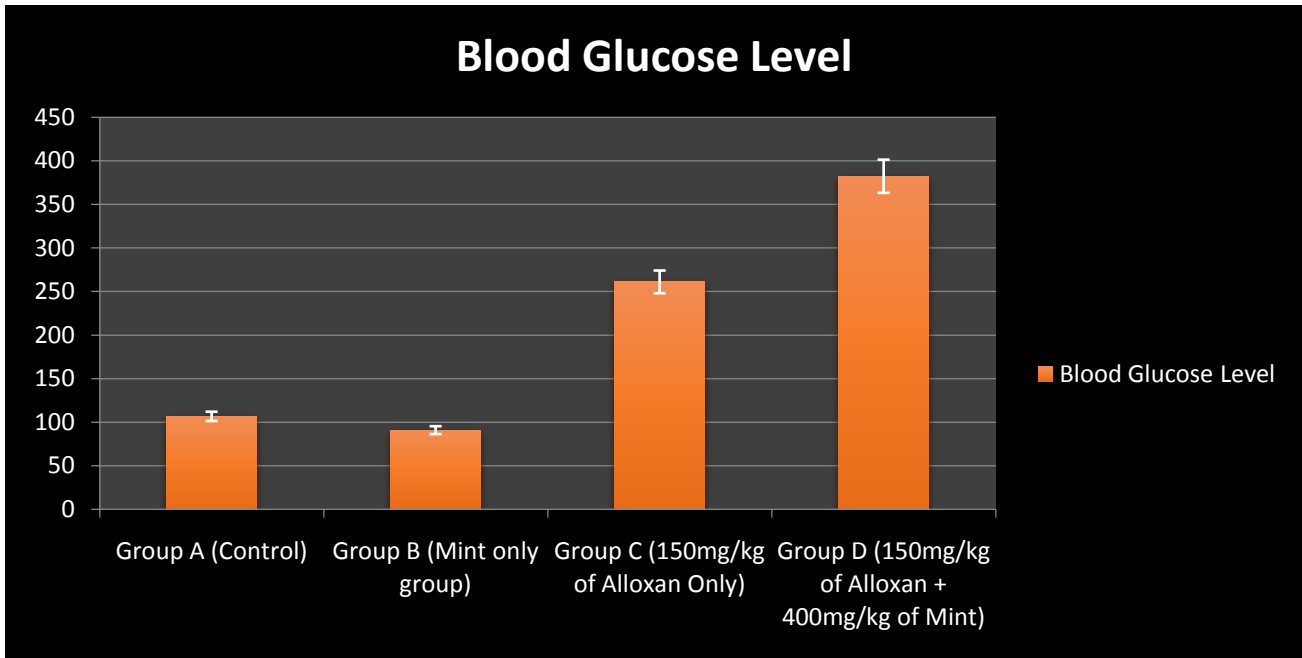


**Fig. 1;** Bar chart shows the initial and final body weights of all groups in various experimental groups studied as represented in table 1

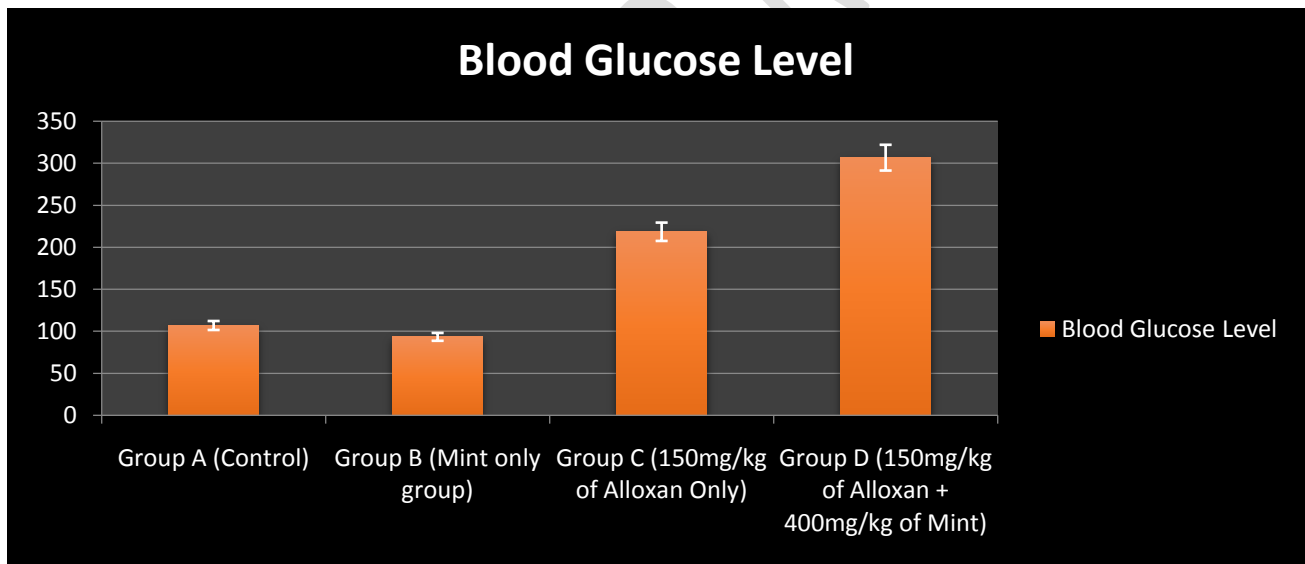
**Table 2:Effect of methanolic leaf extract of *menthapiperita* on the blood glucose levels on alloxan-induced diabetic male wistar rats.**

Weeks	Groups	Mean±SEM	Mean difference	p-Value	f-Value
Week 0	Group A	106.667±4.48	-	-	5.21
	Group B	90.800±5.94	15.8667	.857	
	Group C	261.000±10.94	-154.3333	.109	
	Group D	382.200±120.13	275.5333*	.012*	
Week 2	Group A	106.867±4.28	-	-	1.90
	Group B	93.333±6.22	13.5333	.899	
	Group C	218.400±63.18	-111.5333	.311	
	Group D	306.600±131.27	-199.7333	.089	
Week 4	Group A	115.333±4.40	-	-	1.79
	Group B	92.500±5.79	22.8333	.753	
	Group C	198.667±58.86	-83.3333	.269	
	Group D	231.667±79.44	-116.3333	.136	
Week 6	Group A	119.533±6.27	-	-	1.77
	Group B	91.667±6.56	27.8667	.461	
	Group C	173.333±49.69	-53.8000	.174	
	Group D	129.133±6.43	-9.6000	.797	

**Table 2;** Data was analyzed using a one-way ANOVA test and results were considered significant at  $p < 0.05$ . Results showed non-significant ( $p > 0.05$ ) increase in blood sugar levels in all groups except Group D (diabetic group treated with 400mg/kg of *menthapiperita*) at week 0.

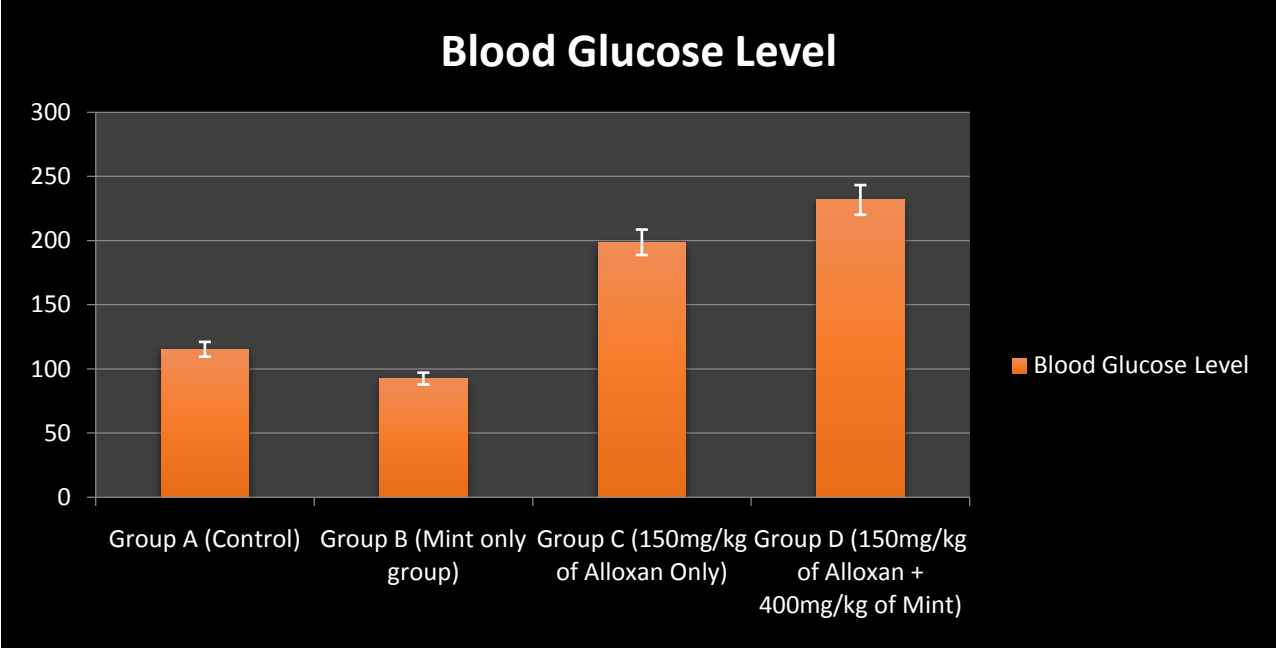


**Fig. 2;** Bar chart showing the blood glucose levels of all experimental groups, confirming type 1

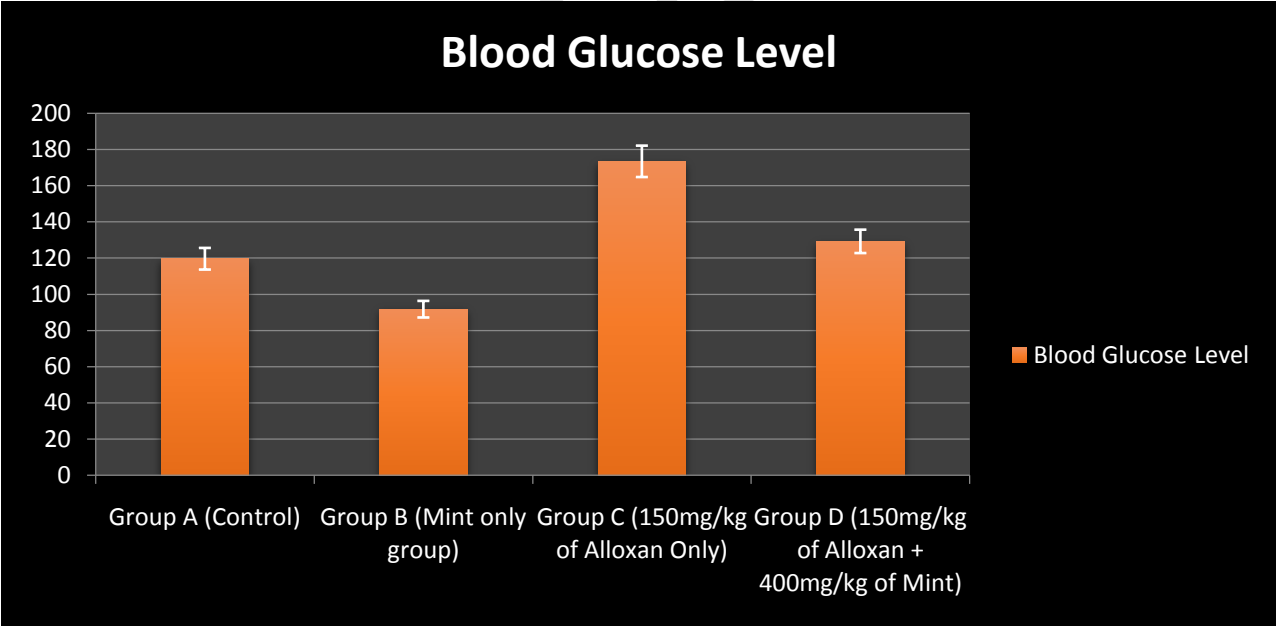


diabetes mellitus studied as represented in table 2

**Fig. 3;** Bar chart showing the blood glucose levels of all experimental groups at week 2 studied and represented in table 2



**Fig. 4;** Bar chart showing the blood glucose levels of all experimental groups at week 4 studied and represented in table 2

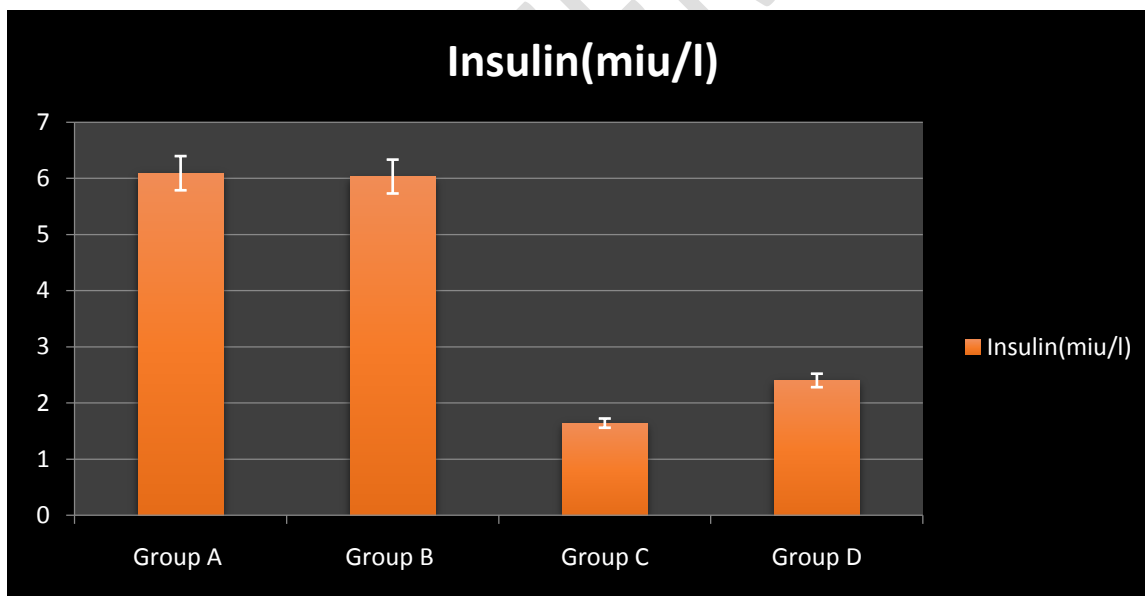


**Fig. 5;** Bar chart showing the blood glucose levels of all experimental groups at week 6 studied and represented in table 2

**Table 3: Effect of methanolic leaf extract of *menthapiperita* on the insulin levels of alloxan-induced diabetic model.**

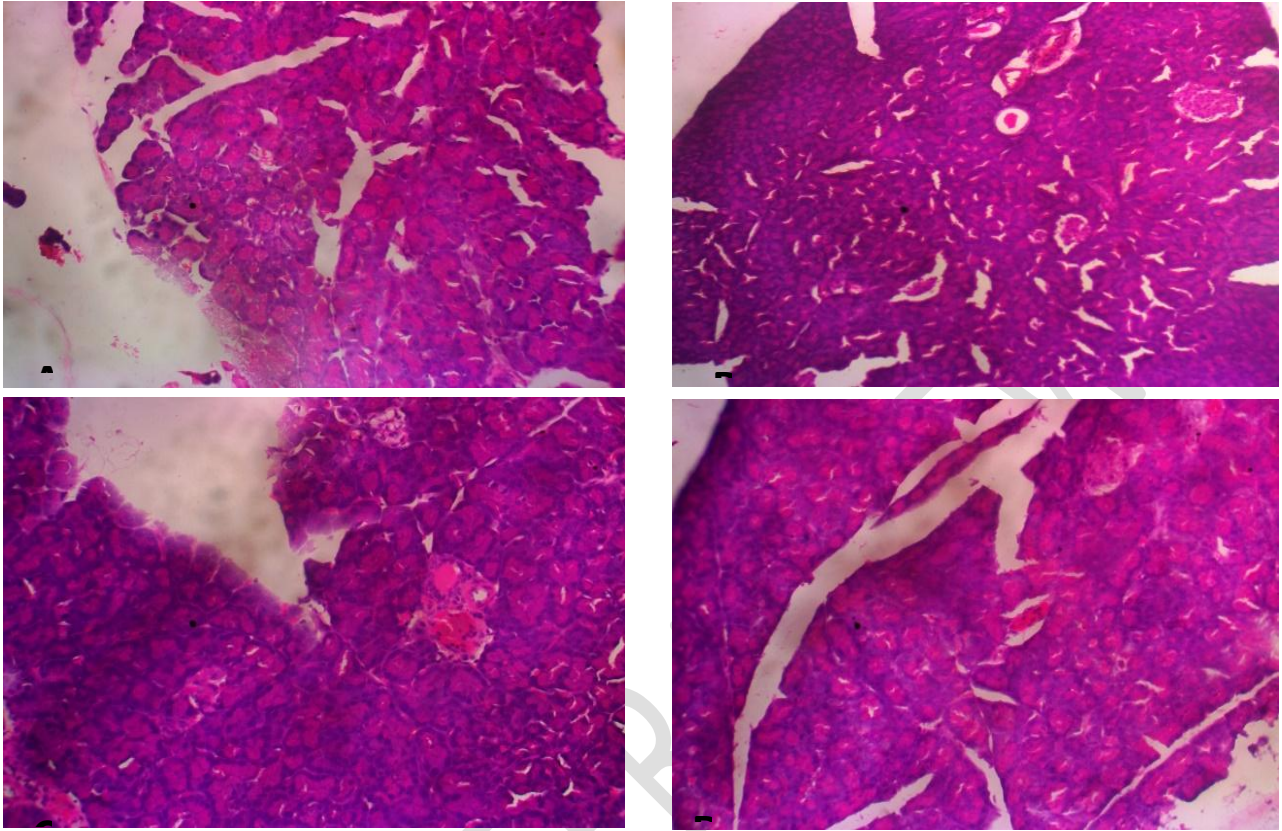
	Groups	Mean±SEM	p-Value
<b>Insulin levels (miu/l)</b>	Group A ( Control; feed and water only)	6.09±0.73	-
	Group B (Mint only)	6.03±0.23	0.92
	Group C (150mg/kg of alloxan monohydrate only)	1.64±0.24	0.000*
	Group D (150mg/kg) of alloxan monohydrate and treated with 400mg/kg of <i>menthapiperita</i> )	2.40±0.34	0.000*

Table 3 showing the serum insulin levels of all experimental groups after administration period. One way ANOVA followed by post HOC fishers LSD multiple comparison was utilised and values were considered significant at  $f(3, 8) = 29.27, p < 0.05$ .



**Fig. 6;** Bar chart showing the insulin levels of all experimental groups after administration in table 3

## Effect of methanolic leaf extract of *menthapiperita* on the histology of the pancreas.



**Plate 1-4:** Plate 1 shows Normal histological features of endocrine pancreas in the control group A (H&E x100). Photomicrograph section of the pancreas shows well-spaced pancreatic acinar cell (PA) and islet of Langerhans (IL) appearing normal; **Plate 2** shows Normal histology showing lobules of predominantly exocrine pancreatic glandular tissue with focal clusters of endocrine cells (arrow). H&E X100. Group B received 400mg/kg of *menthapiperita*; **Plate 3** shows moderate necrosis of the endocrine pancreatic cells, lesions and morphologic alterations in the pancreatic islets of diabetic wistar rats of Group C (alloxan only); **Plate 4** shows lobulated pancreatic tissue with minimally depleted endocrine pancreas cells, showing the anti-oxidant and anti-inflammatory effects of *menthapiperita* on the endocrine pancreas (H&E 100).

### **Conclusion**

The results presented in this study strongly suggests that *menthapiperita* is an effective therapeutic intervention for the treatment of type 1 diabetes mellitus.

### Ethical Approval

The ethical approval for this research was sort and obtained from the ethical committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

## REFERENCES

- Abdellatief, S.A., Beheiry, R.R. and El-Mandrawy, S.A.M. (2017). Peppermint essential oil alleviates hyperglycemia caused by streptozotocin- nicotinamide-induced type 2 diabetes in rats. *Biomedicine & Pharmacotherapy*, 95, pp.990–999. doi:<https://doi.org/10.1016/j.biopha.2017.09.020>.
- Animalab. (n.d.). *Caging systems - plastic cages for laboratory animals and their exploitation*. [online] Available at: <https://animalab.eu/knowledge-base/caging-systems-plastic-cages-for-laboratory-animals-and-their-exploitation> [Accessed 22 May 2023].
- Barbalho, S.M., Damasceno, D.C., Spada, A.P.M., Silva, V.S. da, Martuchi, K.A., Oshiiwa, M., Machado, F.M.V.F. and Mendes, C.G. (2011). Metabolic Profile of Offspring from Diabetic Wistar Rats Treated with *Mentha piperita* (Peppermint). *Evidence-Based Complementary and Alternative Medicine*, 2011, pp.1–6. doi:<https://doi.org/10.1155/2011/430237>.
- Bayani, Mahsan, et al. “Study of Hypoglycemic, Hypocholesterolemic and Antioxidant Activities of Iranian *Mentha Spicata* Leaves Aqueous Extract in Diabetic Rats.” *Iranian*
- Bingham, J.T., Etz, B.D., DuClos, J.M. and Vyas, S. (2021). Structure and Reactivity of Alloxan Monohydrate in the Liquid Phase. *The Journal of Organic Chemistry*, 86(21), pp.14553–14562. doi:<https://doi.org/10.1021/acs.joc.1c01389>.
- Buppajarntham, S. (2020). Insulin: Reference Range, Interpretation, Collection and Panels. *eMedicine*. [online] Available at: <https://emedicine.medscape.com/article/2089224-overview>.
- Dimitriou I, Katsourakis A, Nikolaidou E, Noussios G. The main anatomical variations of the pancreatic duct system: review of the literature and its importance in surgical practice. *J Clin Med Res*. 2018 May. 10(5):370-5. [QxMD MEDLINE Link].
- El Sayed, S.A. and Mukherjee, S. (2020). *Physiology, Pancreas*. [online] PubMed. Available at: <https://pubmed.ncbi.nlm.nih.gov/29083590/>.
- Figueroa-Pérez, M.G., Rocha-Guzmán, N.E., Pérez-Ramírez, I.F., Mercado-Silva, E. and Reynoso-Camacho, R. (2014). Metabolite Profile, Antioxidant Capacity, and Inhibition of Digestive Enzymes in Infusions of Peppermint (*Mentha piperita*) Grown under Drought Stress. *Journal of Agricultural and Food Chemistry*, 62(49), pp.12027–12033.
- Gillespie, D. (n.d.). *The Many Benefits of Disposable Latex Gloves | Unigloves*. [online] [info.unigloves.co.uk](http://info.unigloves.co.uk). Available at: <https://info.unigloves.co.uk/blog/disposable-latex-gloves-benefits#:~:text=Latex%20gloves%20are%20an%20effective>.
- Hamad Al-Mijalli, S., ELsharkawy, E.R., Abdallah, E.M., Hamed, M., El Omari, N., Mahmud, S., Alshahrani, M.M., Mrabti, H.N. and Bouyahya, A. (2022). Determination of Volatile Compounds of *Mentha piperita* and *Lavandulamultifida* and Investigation of Their Antibacterial, Antioxidant, and Antidiabetic Properties. *Evidence-Based Complementary and Alternative Medicine*, 2022, pp.1–9. doi:<https://doi.org/10.1155/2022/9306251>.
- Hamden, K., Ayadi, F., Jamoussi, K., Masmoudi, H. and Elfeki, A. (2008). Therapeutic effect of phytoecdysteroids rich extract from *Ajugaiva* on alloxan induced diabetic rats liver,

- kidney and pancreas. *Biofactors*, [online] 33(3), pp.165–175. Available at: <https://content.iospress.com/articles/biofactors/bio01040> [Accessed 22 May 2023].
- Ighodaro, O.M., Adeosun, A.M. and Akinloye, O.A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, [online] 53(6), pp.365–374. doi:<https://doi.org/10.1016/j.medici.2018.02.001>.
- Ighodaro, Osasenaga Macdonald, et al. “Alloxan-Induced Diabetes, a Common Model for Evaluating the Glycemic-Control Potential of Therapeutic Compounds and Plants Extracts in Experimental Studies.” *Medicina*, vol. 53, no. 6, Jan. 2017, pp. 365–74, doi:<https://doi.org/10.1016/j.medici.2018.02.001>.
- Jain, P., Patale, R., Chaudhary, D., Pawara, Y. and Surana, S. (2016). A REVIEW: ANTI-DIABETIC MEDICINAL PLANTS FROM TORANMAL REGION OF MAHARASHTRA. *Jain et al. World Journal of Pharmaceutical Research*, [online] 5, p.224. doi:<https://doi.org/10.20959/wjpr201610-7078>.
- Journal of Pharmaceutical Research : IJPR*, vol. 16, no. Suppl, 2017, pp. 75–82, [www.ncbi.nlm.nih.gov/pmc/articles/PMC5963648/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5963648/).
- Kostik, V. and Bauer, B. (2015b). *Chemical composition of essential oils of wild-growing Mentha piperita L and Mentha spicata L from the Mariovo region, Republic of Macedonia*. [online] [eprints.ugd.edu.mk](http://eprints.ugd.edu.mk). Available at: <http://eprints.ugd.edu.mk/13112/> [Accessed 22 May 2023].
- Lefèbvre, Pierre J., and Daniel G. Pipeleers, editors. *The Pathology of the Endocrine Pancreas in Diabetes*. Springer Berlin Heidelberg, 1988, doi:<https://doi.org/10.1007/978-3-642-72691-0>.
- Leighton, E., Sainsbury, C.A. and Jones, G.C. (2017). A Practical Review of C-Peptide Testing in Diabetes. *Diabetes Therapy*, 8(3), pp.475–487. doi:<https://doi.org/10.1007/s13300-017-0265-4>.
- Lenzen, S. “The Mechanisms of Alloxan- and Streptozotocin-Induced Diabetes.” *Diabetologia*, vol. 51, no. 2, Dec. 2007, pp. 216–26, doi:<https://doi.org/10.1007/s00125-007-0886-7>.
- Li YL, Yu ML, Lee KH. The crossing duct sign. *AbdomRadiol (NY)*. 2018 Jun. 43(6):1506-7. [QxMD MEDLINE Link].
- Longnecker, D. (2019). Anatomy and Histology of the Pancreas. *The Pancreapedia: Exocrine Pancreas Knowledge Base*. [online] doi:<https://doi.org/10.3998/panc.2014.3>.
- Lybrate. (n.d.). *Hormonal Assay Test - Test Results, Normal Range, Cost And More*. [online] Available at: <https://www.lybrate.com/lab-test/hormonal-assay> [Accessed 29 May 2023].
- Malaisse, Willy J. “Alloxan Toxicity to the Pancreatic B-Cell: A New Hypothesis.” *Biochemical Pharmacology*, vol. 31, no. 22, Nov. 1982, pp. 3527–34, doi:[https://doi.org/10.1016/0006-2952\(82\)90571-8](https://doi.org/10.1016/0006-2952(82)90571-8).
- Mani Badal, Rajendra, et al. “Pharmacological Action of Mentha Piperita on Lipid Profile in Fructose-Fed Rats.” *Iranian Journal of Pharmaceutical Research : IJPR*, vol. 10, no. 4, 2011, pp. 843–48,

- Manohar, M., Verma, A.K., Venkateshaiah, S.U., Sanders, N.L. and Mishra, A. (2017). Pathogenic mechanisms of pancreatitis. *World Journal of Gastrointestinal Pharmacology and Therapeutics*, [online] 8(1), pp.10–25. doi:<https://doi.org/10.4292/wjgpt.v8.i1.10>.
- Mayo Clinic. (2016). *Blood glucose meter: How to choose*. [online] Available at: <https://www.mayoclinic.org/diseases-conditions/diabetes/in-depth/blood-glucose-meter/art-20046335>.
- Mayo Clinic. “Diabetes - Symptoms and Causes.” *Mayo Clinic*, 20 Jan. 2023, [www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444](http://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444).
- Medical Encyclopedia: Insulin test. (n.d.). Available at: <https://www.hsc.wvu.edu/media/5198/insulin-summary.pdf> [Accessed 22 May 2023].
- Medlineplus.gov. (2018). *C-Peptide Test: MedlinePlus Lab Test Information*. [online] Available at: <https://medlineplus.gov/lab-tests/c-peptide-test/>.
- medlineplus.gov. (n.d.). *Insulin in Blood: MedlinePlus Medical Test*. [online] Available at: <https://medlineplus.gov/lab-tests/insulin-in-blood/#:~:text=What%20is%20an%20insulin%20in.>
- Mulholland MW, Moosa AR, Liddle RA. Pancreas: anatomy and structural anomalies. In: Yamada T, ed. *Textbook of Gastroenterology*. Philadelphia, Pa: JB Lippincott; 1995.
- National Institute of Diabetes and Digestive and Kidney Diseases (2019). *Insulin Resistance & Prediabetes | NIDDK*. [online] National Institute of Diabetes and Digestive and Kidney Diseases. Available at: <https://www.niddk.nih.gov/health-information/diabetes/overview/what-is-diabetes/prediabetes-insulin-resistance>.
- NHS Choices. “Type 1 Diabetes.” *NHS*, 2019, [www.nhs.uk/conditions/type-1-diabetes/](http://www.nhs.uk/conditions/type-1-diabetes/).
- Oyeleye, S.I., Adefegha, A.S., Ojo, O.R., Akintemi, O.A. and Ganiyu Oboh (2023). Ameliorative effect of extracts from scent (*Ocimum basilicum*), mentha (*Mentha piperita*), and thyme (*Thymus vulgaris*) leafy spices on glycemc, purinergic molecules, and oxidative status in renal tissue of diabetic rats. doi:<https://doi.org/10.1007/s00580-023-03476-z>.
- P, V., E, D. and Ks, S. (2021). ENZYMATIc AND NON-ENZYMATIc ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT OF MENTHA PIPERITAL. *Journal of Advanced Scientific Research*, [online] 12(01 Suppl 1), pp.140–144.
- P, Valsala, et al. “ENZYMATIc AND NON-ENZYMATIc ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT OF MENTHA PIPERITAL.” *Journal of Advanced Scientific Research*, vol. 12, no. 01 Suppl 1, Mar. 2021, pp. 140–44, [sciensage.info/index.php/JASR/article/view/1110](http://sciensage.info/index.php/JASR/article/view/1110).
- Park, B. H., et al. “Protective Mechanism of Glucose against Alloxan-Induced Pancreatic  $\beta$ -Cell Damage.” *Biochemical and Biophysical Research Communications*, vol. 210, no. 1, May 1995, pp. 1–6, doi:<https://doi.org/10.1006/bbrc.1995.1619>.
- Patil, S.R., Patil, R.S. and Godghate, A.G. (2016). MENTHA PIPERITA LINN: PHYTOCHEMICAL, ANTIBACTERIAL AND DIPTERIAN ADULTICIDAL APPROACH. *International Journal of Pharmacy and Pharmaceutical Sciences*, [online]

- pp.352–355. Available at: <https://journals.innovareacademics.in/index.php/ijpps/article/view/10370/4868#:~:text=R%20The%20Mentha%20piperita%20were> [Accessed 26 May 2023].
- Pet, P. (n.d.). *Best Litter For Rats: What You Need To Know*. [online] Planet Pet. Available at: <https://www.planetpet.com.au/blog/best-litter-for-rats> [Accessed 22 May 2023].
- Pharmacognosy - Medicinal Plants. (n.d.). *Infusion*. [online] Available at: <https://www.medicinalplants-pharmacognosy.com/pharmacognosy-s-topics/extraction-methods/infusion/>.
- Rad, Adrian. “Pancreas.” *Kenhub*, 22 July 2022, [www.kenhub.com/en/library/anatomy/the-pancreas](http://www.kenhub.com/en/library/anatomy/the-pancreas).
- Rahman, S., Jan, G., Jan, F.G. and Rahim, H.U. (2022). Phytochemical Analysis and hypoglycemic potential of *Filagohurdwarica* (Wall. ex DC.) Wagenitz in alloxan induced diabetic mice. *Brazilian Journal of Biology*, [online] 84, p.e261518.
- Randhawa, Muhammad. “CALCULATION of LD 50 VALUES from the METHOD of MILLER and TAINTER, 1944.” *J Ayub Med Coll Abbottabad*, vol. 21, no. 3, 2009, [ayubmed.edu.pk/JAMC/PAST/21-3/Randhawa.pdf](http://ayubmed.edu.pk/JAMC/PAST/21-3/Randhawa.pdf).
- Shahein, Magdy Ramadan, et al. *The Biological Effects of Fermented Camel Milk Fortified with Sage (Salvia Officinalis L.) and Mint (Mentha Piperita) Leaves Powder on Alloxan-Induced Diabetic Rats*. Feb. 2022, doi:<https://doi.org/10.21203/rs.3.rs-1362024/v1>.
- Talathi, S.S., Zimmerman, R. and Young, M. (2023). *Anatomy, Abdomen and Pelvis, Pancreas*. [online] PubMed. Available at: <https://pubmed.ncbi.nlm.nih.gov/30422507/#:~:text=The%20pancreas%20is%20an%20extended> [Accessed 22 May 2023].
- Testing.com. (2021). *Insulin Test*. [online] Available at: <https://www.testing.com/tests/insulin/>.
- Toghyani, Mehdi, et al. “Growth Performance, Serum Biochemistry and Blood Hematology of Broiler Chicks Fed Different Levels of Black Seed (*Nigella Sativa*) and Peppermint (*Mentha Piperita*).” *Livestock Science*, vol. 129, no. 1-3, Apr. 2010, pp. 173–78, doi:<https://doi.org/10.1016/j.livsci.2010.01.021>.
- Wang, J.-J., Jin, H., Zheng, S.-L., Xia, P., Cai, Y. and Ni, X.-J. (2017). Phytoecdysteroids from *Ajugaiva* act as potential antidiabetic agent against alloxan-induced diabetic male albino rats. *Biomedicine & Pharmacotherapy*, [online] 96, pp.480–488. doi:<https://doi.org/10.1016/j.biopha.2017.10.029>.
- Wikipedia Contributors (2019). *Methanol*. [online] Wikipedia. Available at: <https://en.wikipedia.org/wiki/Methanol>.
- Wilcox, G. (2005). Insulin and Insulin Resistance. *The Clinical biochemist. Reviews*, [online] 26(2), pp.19–39. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1204764/>.
- World Health Organization (2022). *Diabetes*. [online] World Health Organization. Available at: [https://www.who.int/health-topics/diabetes#tab=tab\\_1](https://www.who.int/health-topics/diabetes#tab=tab_1).

www.portea.com. (n.d.). *Insulin Fasting Test - Normal Range, Procedure, Results & More.* [online] Available at: <https://www.portea.com/labs/diagnostic-tests/insulin-fasting-test-225/>.

www.sciencedirect.com. (n.d.). *Mentha piperita - an overview | ScienceDirect Topics.* [online] Available at: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/mentha-piperita#:~:text=Mentha%20piperita%20is%20a%20hybrid>.

www.wbdg.org. (n.d.). *Animal Research Facility | WBDG - Whole Building Design Guide.* [online] Available at: <https://www.wbdg.org/building-types/research-facilities/animal-research-facility#:~:text=The%20animal%20research%20facility%2C%20also>.

Yin, P., Wang, Y., Yang, L., Sui, J.-L. and Liu, Y. (2018). Hypoglycemic Effects in Alloxan-Induced Diabetic Rats of the Phenolic Extract from Mongolian Oak Cups Enriched in Ellagic Acid, Kaempferol and Their Derivatives. [online] 23(5), pp.1046–1046. doi:<https://doi.org/10.3390/molecules23051046>.

Jaykaran, Yadav, P. and Kantharia, N. (2011). Reporting of method of animal sacrifice in articles published in Indian journals. *Journal of Pharmacology and Pharmacotherapeutics*, 2(2), p.125. doi:<https://doi.org/10.4103/0976-500x.81912>.

Medscape.com. (2019). *Exocrine Pancreatic Insufficiency: Practice Essentials, Anatomy, Pathophysiology.* [online] Available at: <https://emedicine.medscape.com/article/2121028-overview>.

www.bionity.com. (n.d.). *Mentha.* [online] Available at: <https://www.bionity.com/en/encyclopedia/Mentha.html> [Accessed 21 Jun. 2023].

Toppr-guides. (2018). *Toppr.com.* [online] Available at: <https://www.toppr.com/guides/biology/plant-kingdom/bryophytes/>.

Trevisan, S., Menezes, A.P., Barbalho, S. and Guiguer, É. (2017). *PROPERTIES OF MENTHA PIPERITA: A BRIEF REVIEW.* [online] [www.semanticscholar.org](http://www.semanticscholar.org).

Available at: <https://www.semanticscholar.org/paper/PROPERTIES-OF-MENTHA-PIPERITA%3A-A-BRIEF-REVIEW-Trevisan-Menezes/35cca1e283fe398609e3dff647f1d9c2abadb6eb>.

Ighodaro, O.M., Adeosun, A.M. and Akinloye, O.A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, [online] 53(6), pp.365–374. doi:<https://doi.org/10.1016/j.medici.2018.02.001>.

Toppr-guides. (2018). *Toppr.com.* [online] Available at: <https://www.toppr.com/guides/biology/plant-kingdom/bryophytes/>.

Trevisan, S., Menezes, A.P., Barbalho, S. and Guiguer, É. (2017). *PROPERTIES OF MENTHA PIPERITA: A BRIEF REVIEW*. [online] [www.semanticscholar.org](http://www.semanticscholar.org). Available at: <https://www.semanticscholar.org/paper/PROPERTIES-OF-MENTHA-PIPERITA%3A-A-BRIEF-REVIEW-Trevisan-Menezes/35cca1e283fe398609e3dff647f1d9c2abadb6eb>.

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