Original Article

Eugenia uniflora L. Leaf Extract Ameliorated Metabolic Traits and Reduced Visceral Adiposity in Rat Model of Metabolic Syndrome

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ABSTRACT

Background

The prevalence of metabolic syndrome has risen globally in recent years. Visceral fat and insulin resistance have been reported as crucial factors for chronic inflammation that caused several complications of metabolic syndrome. Pitanga (Myrtaceae) are extensively dispersed and have long been utilized as herbal treatments and reported to have antioxidant and antidiabetic activities.

Objective

Investigate the activities of *Eugenia uniflora* L. leaf extract on the metabolic syndrome components; epididymal fat and pancreas histology in a rat model.

Method

The experiment followed a post-test only design using 25 male Wistar rats. The animals were randomly allocated into five equal groups: normal, metabolic syndrome (MS), MS + telmisartan 8 mg/kg as standard treatment; MS + *Eugenia uniflora* L. leaf extract (ELE) 50 mg/kg; and MS + ELE 100 mg/kg. Metabolic syndrome was induced by high-fat diets and 20% fructose in drinking water for 8 weeks simultaneously with standard treatment and extracts. At day 57, metabolic syndrome components were measured, and epididymal fat as well as the pancreas were taken for histological analysis.

Results

Administration of ELE 100 mg/kg improved metabolic syndrome components, including reduced blood glucose and triglycerides levels; reduced systolic blood pressure and Lee Index, also increased HDL-cholesterol levels. The ELE 100 mg/kg also reduced epididymal adipocyte area, and the result was comparable with telmisartan. However, neither telmisartan nor ELE affected the size of pancreatic islets.

Conclusion

ELE improves metabolic syndrome components and reduces visceral adiposity in rat models induced by HFD and fructose combination.

Keyword

metabolic syndrome; Eugenia, high-fat diet; pancreas; adipocyte

INTRODUCTION

Metabolic syndrome (MS) is introduced in 1988 by Reaven and colleagues, and can be elucidated as a constellation of various metabolic disorders, such as obesity, glucose intolerance, elevated triacylglycerol level, low HDL-cholesterol level, and elevated blood pressure[1–3]. MS substantially increases the risk of cardiovascular and cerebrovascular diseases, as well as type 2 diabetes mellitus[4]. People with MS are also more prune to develop organ-related abnormalities, including non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), along with various organ dysfunction, infertility, dementia, and obstructive sleep disorders[1,3–5]. Moreover, MS is correlated with an increased incidence, morbidity, and mortality of cancers involving the liver, pancreas, cervix, and breast[1,6,7].

The global prevalence of MS grows at an alarming rate, now ranges from 20% to 30% of the world's adult population[8,9]. The prevalence is expected to escalate due to increasing prevalence of obesity, excessive consumptions of calorie-dense foods and sugary drinks, as well as sedentary lifestyles[2,10]. Visceral obesity plays an essential factor for chronic inflammation, hyperlipidemia, insulin resistance, and cardiovascular disorders[11]. Visceral adipocytes are typically larger in size and release several pro-inflammatory mediators, including prostaglandins, C-reactive protein,

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and various cytokines, which contribute to an increased risk of metabolic disorders including type 2 diabetes mellitus, hyperlipidemia, and cardiovascular disease[12,13].

Eugenia uniflora L. (Myrtaceae) is a native Brazilian plant that grows broadly in various tropical regions and has been conventionally used for its antidiabetic, antidiarrheal, diuretic, and antihypertension properties[14,15]. Several studies also highlighted its potential antioxidant, antimicrobial, cytoprotective, nephroprotective, and anti-neoplastic effects[16–19]. Among its plant parts, the fruits and leaves of *E. uniflora* have been extensively investigated[15]. Given the limited availability of the fruit and the abundance of leaves with promising pharmacological potential, this study aims to investigate the activity of *E. uniflora* leaf extract on metabolic parameters, visceral fat, and pancreatic histology in a rat model of metabolic syndrome.

MATERIALS AND METHODS

Extract preparation

About 500 g of fresh leaves of *E. uniflora* were gathered in April 2024 from a tree planted in Sleman, Yogyakarta, Indonesia (GPS coordinates -7.703718/S7°42'13.387", 110.427723/E110°25'39.801"). Plant specimen was collected in the herbarium and identified by the Faculty of Biology, Gadjah Mada University (00608/S.Tb/IV/2024). The leaves were dried at 40°C and ground to obtain 300 g of powder. All simplisia were then soaked with 5 L of 96% ethanol for 3 days and then filtered. All filtrates were then evaporated by rotary evaporation (50 °C; 178 mbar; 60 rpm), resulting in the 48.05 g crude extract (16.02% yield)[17]. The extraction was executed at the Pharmaceutical Biology Laboratory, Islamic University of Indonesia.

Experimental animals and intervention

Twenty-five male *Rattus norvegicus* (Wistar strain) aged 8-10 weeks and weighing 175 ± 25 g were used in this study. The number of animals was determined according to the Festing formula for experimental design[20]. Prior to treatment, all animals were acclimatized for seven days under standard laboratory conditions. They were maintained in group cages with controlled environmental parameters, including 12 hours dark/light cycle, ambient temperature 22 ± 3°C, approximately 40% relative humidity. The experiment was executed at the Integrated Research Laboratory, Islamic University of Indonesia. Rats had free access to standard chow and tap water for seven days of adaptation and then randomly assigned into five groups using a random number generator: control, untreated metabolic syndrome model (MS), MS received telmisartan 8 mg/kg as standard treatment (MS+TMS), MS received ELE 50 mg/kg (MS+ELE50), and MS received ELE 100 mg/kg (MS+ELE100). Metabolic syndrome was induced by gavaging an oxidized oil and quail egg yolk combination (1:2) and 20% D-fructose as drinking water (FDW) (Merck Milipore, Indonesia) for 56 days[21,22]. All treatment regimens and doses were determined and given simultaneously as an oral solution not exceeding 1 ml/g/day every 8 a.m (Figure 1)[23,24].

Anthropometric and Blood Pressure Measurements

The nasoanal length of each rat was determined by measuring the distance from the tip of the nose to the anus using a calibrated graph. Body weight was recorded with a calibrated digital scale (Quattro; Indonesia). Lee indexes were calculated according to the following formula:[25]

$$Lee\ index = \frac{\sqrt[3]{Body\ weight\ (g)}}{Nasoanal\ length\ (cm)} \times 1{,}000$$

Blood pressures were recorded using a non-invasive blood pressure system (Panlab LE 5002, Harvard Apparatus; Canada) following a two-day adaptation period to the apparatus. Three consecutive readings were taken for each rat and the mean value was considered for analysis.

Biochemical Parameter Measurements

On day 57, all rats were anesthetized with an intraperitoneal injection of ketamine (25 mg/kg; Sandoz, Canada) following 12-h fasting period. Blood samples was collected from the retro-orbital plexus and centrifuged at 1,500 x g for 15 min to prepare plasma, which was preserved at -20°C until analysis. Biochemical parameters were determined using commercial assay kits (Wako Pure Chemical Industries, Japan)[25].

Histological Preparation and Examination

After all animals were euthanized using the guillotine, a longitudinal incision was performed, and internal organs were visualized. Pancreas and adipose tissue surrounding the testis and epididymis were taken[26].

Epididymal fat was processed into 5 µm paraffin-tissue sections, deparaffinized, and stained with hematoxylin-eosin (HE). Adipocyte tissue was determined in five representative photomicrographs taken from a CX22 light microscope (Olympus, Japan) equipped with Optilab Viewer imaging system (Miconos, Indonesia). Adipocyte area (µm²) was quantified with ImageJ FIJI software, version 2.0 (National Institutes of Health, Bethesda, MD, USA) at 10X magnification using the Adiposoft plugin version 1.16 using manual edition and exclusion of the cell located at the edge to enhance accuracy[27–29]. Pancreatic islet parameters were measured on five representative fields at 400X magnification. The diameter was taken twice, at the maximum transverse diameter of islets and perpendicular to the previous. The transvertical diameter were calculated based on the Formula[30]:

$$Transvertical\ diameter = \frac{(maximum\ transverse\ diameter + maximum\ perpendicular\ diameter)}{2}$$

The measurement was performed independently by two observers blinded to the treatment, and the Intraclass Correlation Coefficient (ICC) value was 0.96, indicating an excellent agreement (ICC value > 0.90)[31].

Statistical analysis

Statistical analysis were performed with GraphPad software version 9.3.1. Data normality was verified using the Shapiro-Wilk test. All result were presented as mean ± standard deviation. Group differences were assessed by one-way ANOVA followed by appropriate post hoc comparisons, and p velues below 0.05 were considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, Islamic University of Indonesia (5/Ka.Kom.Et/70/KE/XII/2023).

RESULTS

E. uniflora extract and telmisartan improved the metabolic syndrome component

As shown in Figure 2, treatments exerted distinct effects on the components of MS. The MS group demonstrated a significant elevated glucose levels compared with controls. Treatment with 100 mg/kg ELE effectively lowered glucose levels, comparable to the effect observed with telmisartan. In addition, both systolic and diastolic blood pressure were significantly higher in the MS group. Notably, ELE 100 mg/kg significantly decreased systolic blood pressure similar with telmisartan's effect. However, we did not find a similar effect on diastolic blood pressure. The Lee index, as a surrogate marker of obesity in rodents, increased in the MS group, and ELE treatment attenuated this in a dose-dependent manner. The triglyceride levels were significantly elevated in the MS group. Treatment with ELE 100 mg/kg significantly reduced triglyceride levels comparable to telmisartan. HDL-cholesterol was also reduced in the MS group. Administration of 100 mg/kg ELE led to a significant increase in HDL levels, restoring them closer to control value.Taken together, the data in Figure 2 indicate that ELE exerts beneficial effects on MS components, comparable to telmisartan.

E. uniflora leaf extract and telmisartan reduced epididymal adiposity.

Figure 3 illustrates the effect of treatments on epididymal fat; panels A–E present histological sections, while panel F quantitatively summarizes the mean fat cell area for each group. The control group shows tightly packed adipocytes of relatively small and uniform size. In contrast, the MS group reveals enlarged adipocytes with significant hypertrophy. Treatment with telmisartan resulted in a marked reduction in adipocyte size compared with the MS group. A comparable, though moderate, decrease in adipocyte size was also observed in the MS + ELE50 group. Notably, panel E displays adipocyte morphology resembling that of the control group, characterized by diminished hypertrophy and improved cellular uniformity. Quantitative analysis of fat area (panel F) revealed a significant enlargement of adipocyte in the MS group relative to the control. Both telmisartan and ELE at 50 mg/kg and 100 mg/kg significantly decreased fat area compared with the MS group, with the higher ELE dose (100 mg/kg) producing an effect comparable to telmisartan.

E. uniflora leaf extract and telmisartan attenuated pancreatic damage

Figure 4 presents histopathological evidence of the pancreas. Image A depicts a pancreas from the control group, showing intact pancreatic acini and well-preserved pancreatic islets. In contrast, the MS group reveals pronounced pathological alterations, including extensive acinar and insular vacuolization, adipocyte infiltration, chronic inflammation, and fibrosis. The MS + ELE50 group demonstrates notable abnormalities, although the severity appears attenuated. Panels D and E are higher magnifications of the boxed regions in B, highlighting pancreatic injury in the MS group, including adipose infiltration, chronic inflammation, fibrotic areas, and multiple vacuolated islet cells and adjacent adipocytes. Panel F further emphasizes insular vacuolization in the MS + ELE50 group, demonstrating clear disruption of islet integrity and insular vacuolization. The lower panel of the figure provides a qualitative summary of the pathological changes across groups. The MS group shows acinar and insular vacuolization along with inflammation and fibrosis. Treatment with telmisartan ameliorated these changes, as evidenced by the absence of pathological features. The MS + ELE50 group displayed acinar and insular vacuolization, but no signs of inflammation or fibrosis, indicating partial protection. Interestingly, the MS + ELE100 group showed only mild insular vacuolization, suggesting a dose-dependent protective effect of ELE against MS-induced pancreatic injury.

E. uniflora leaf extract and telmisartan did not affect pancreatic islet diameter and area

The upper panels (A–F) in Figure 5 illustrates pancreatic morphological differences, while the lower panel quantifies pancreatic islet diameter and area. Panel A displays normal and compact pancreatic islet structure surrounded by intact acinar cells (control group). In contrast, panels B and C reveal signs of islet and acinar vacuolization observed in the MS group. Panel D and E show reduced insular vacuolization, suggesting partial recovery or attenuation of the pancreatic damage with either telmisartan or low-dose ELE treatment. Notably, panel F (MS + ELE100) shows a largely preserved pancreatic islet with normal morphology. However, the quantitative analysis in the lower panel indicates no differences in pancreatic islet diameter or area among groups. These findings suggest that while metabolic syndrome induces cellular-level changes such as vacuolization, it does not significantly affect islet size.

Discussion

This current research highlights the potencial of *E. uniflora* leaf extract in mitigating metabolic abnormalities, visceral adiposopathies, as well as pancreatic changes in a rat model of metabolic syndrome. The findings indicate that MS induces significant disturbances in metabolic parameters as well as in the morphology of pancreatic and adipose tissues, all of which were ameliorated by ELE, particularly at the dose of 100 mg/kg.

Our study reported that the HFD and 20% FDW significantly induced MS traits such as hyperglycemia, hypertriglyceridemia, hypo-HDL-cholesterolemia, hypertension, and obesity. Previous research have demonstrated alterations in clinical parameters--including elevated fasting glucose, triglyceride levels, and body weight relevant--in animals fed a HFD combined with fructose-drinking water, reflecting metabolic syndrome conditions. This model has also been shown to increase the adiposity index, induce insulin resistance, elevate pro-inflammatory cytokines, and cause histomorphometric alteration in the liver, pancreas, and adipose tissue[32,33]. Compared with other methods, this combination of diets has several advantages, even though HFD and fructose alone may cause changes in metabolic and target-organ abnormalities[34,35]. The model closely reflects current eating habits trend in the society, where HFD contribute to obesity, and excessive fructose intake leads to elevated triglyceride levels. Such a diet also decreases HDL-C and elevates plasma cholesterol, representing a typical pattern of dyslipidemia[34–37].

Obesity traits in metabolic syndrome were also reported to promote adipocytes' hypertrophy and pancreatic steatosis[32,38]. HFD is usually accompanied by insulin resistance, resulting in cellular condensation and atrophy, inflammation, and peri-insular fibrosis[34]. Insulin resistance plays a crucial role in promoting lipid accumulation within both adipose and the pancreas, consequently leading to chronic inflammation[39,40]. The accumulation of visceral fat will undergo lipolysis and increase the supply of free fatty acids to the target organs[41]. This condition can lead to cytoplasm vacuolization, irregularity in β-cells' nuclear shape, and nucleolar segmentation, while in α-cells, MS can lead to increased glucagon expression, chromatin condensation, and diminished cell boundaries [39]. On the

other hand, increased blood glucose levels and free fatty acids can trigger β-cell hyperplasia and hypertrophy via expression of cyclin-D2 activated by the protein kinase C (PKC)-phosphatidyl inositol-3 kinase (PI3K) pathway and cause the size of the islets of Langerhans to enlarge[42,43].

Both telmisartan and ELE reduced these clinical and microstructural abnormalities, with the higher dose of ELE showing efficacy comparable to telmisartan, particularly in reducing blood glucose, triglycerides, and systolic blood pressure. This finding is consistent with previous reports demonstrating the protective effect of *E. uniflora* leaf extract, obtained through various extraction methods, in both *in vitro and in vivo* models of MS. *E. uniflora* leaf extract has been reported to orchestrate enzymatic processess related to metabolism, exhibit antioxidant activity, and reduce glycation in *in vitro* studies. In *in vivo* experiments, the extract demonstrated protective effects characterized by enhanced insulin secretion and insulinotropic activity; increased serum antioxidant capacity; reduced serum glucose levels, elevated HDL-cholesterol; and decreased lipid parameters. Phytochemical analyses have revealed that the extract contains various bioactive constituents, such as alkaloids, flavonoids, phenolic components—primarily ellagic acid, gallic acid, rutin, quercetin, and valoneic acid), myricetin, terpenes, tannins, and phenolic acids[44]. Other studies have reported the clinical benefits and antioxidant effects of chronic administration of ELE in non-obese diabetic mice, as evidenced by the maintainance of normal serum insulin levels, reduced serum lipid peroxidation, and protection of target organs such as the liver and pancreas. These protective effect were further supported by a decreased inflammatory index in the pancreatic islets of Langerhans and an increased in hepatic glutathione levels[45].

Earlier research demonstrated that *E. uniflora* extract exerts protective actions in rats subjected to a high-palatable diet-induced model of metabolic syndrome. The extract reduced serum IL-6 levels and enzymes responsible for inflammatory and thromboregulatory modulation, such as adenosine deaminase, nucleoside triphosphate diphosphohydrolases, acetylcholinesterase, and butyrylcholinesterase[46]. Similar results were also reported on metabolic alterations and oxidative markers of the dexamethasone-induced insulin-resistance rat model[47].

Phytochemical analysis revealed that *E. uniflora* leaf extracts were rich in flavonoids, phenols, saponins, and tannins, and the extract also exhibited very strong antioxidant activity in ABTS *in vitro* methods[48]. Phenolic compounds contained in *E. uniflora* extract, such as quercetin, provide antioxidant effects by scavenging free radicals produced from lipid peroxidation. The benefit of *E. uniflora* extract administration was not limited to the metabolic alteration but also affected the neurostructure of the hippocampus and striatum, improving neurochemical parameters and behavioral parameters of the rats[49]. Analysis by high-performance liquid chromatography with photodiode array detection revealed that the crude extract and fractionated extract of *E. uniflora* leaves contain several bioactive constituents, including gallic acid, ellagic acid, and myricitrin. These extract demonstrated notable antibacterial, anti-inflammatory, and antioxidant activities[50].

This current study has several limitations. First, the direct molecular mechanisms have not been investigated yet. Second, the study did not evaluate insulin levels or glucose tolerance, which would provide insight into β-cell function and insulin sensitivity, and finally, long-term safety and pharmacokinetic profiles of *E. uniflora* extract were not assessed yet.

CONCLUSION

In conclusion, *Eugenia uniflora* leaf extract effectively ameliorated multiple components of MS in rats, including metabolic disturbances, adipocyte hypertrophy, and pancreatic damage. Its therapeutic effect, particularly at 100 mg/kg, was comparable to telmisartan in many respects, supporting the potential of *E. uniflora* leaf extract as a natural adjunctive therapy for MS, although further studies are required to clarify its molecular mechanisms and long-term safety in clinical settings.

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Conflicts of interest

None to declare.

Ethical Clearance

Ethical clearance was obtained from the Health

Research Ethics Committee, Faculty of Medicine, Islamic University of Indonesia (5/Ka.Kom.Et/70/KE/XII/2023).

Declaration of Use of AI in Scientific Writing

During the manuscript preparation, the authors used QuillBot to correct any grammatical errors and free version of Biorender to create the image (https://www.biorender.com/). After using this tool/service, the authors reviewed and edited the content as required.

Authors' Contributions

Data gathering and idea owner of this study: Evy Sulistyoningrum

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Writing and submitting manuscript: Dede Syifa Izzatul Aulia, Evy Sulistyoningrum

Editing and approval of final draft: Dede Syifa Izzatul Aulia, Evy Sulistyoningrum

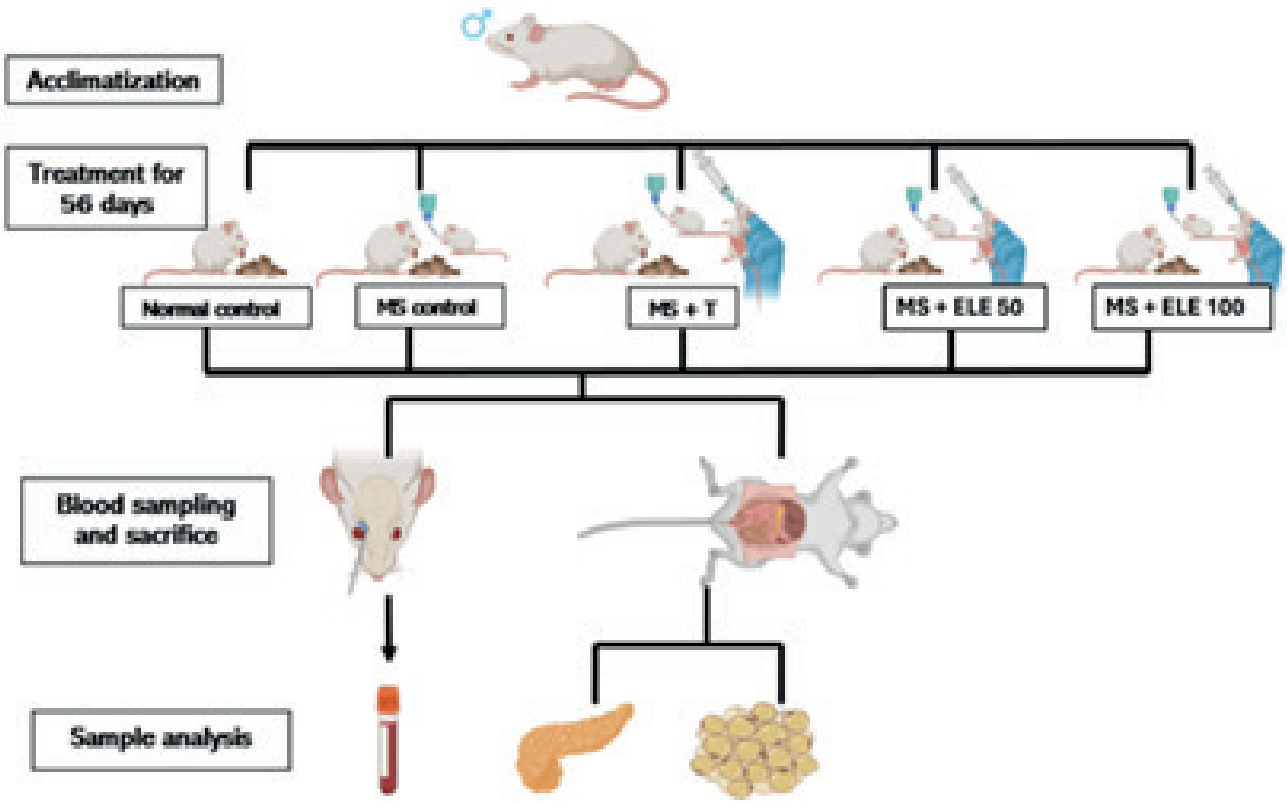


Figure 1. Schematic diagram of the research intervention

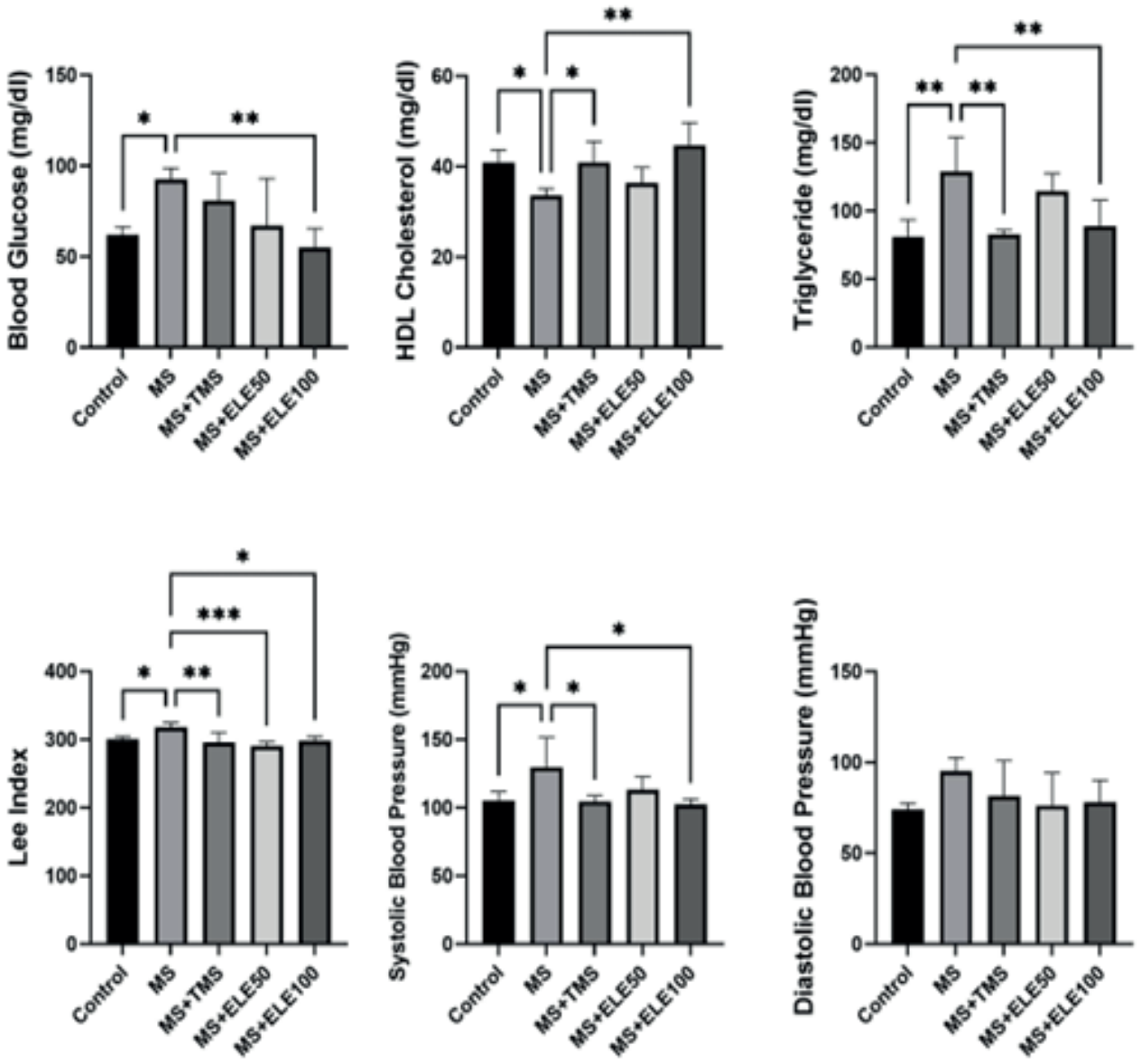


Figure 2. Effect of *E.uniflora* leaf extract on the metabolic syndrome components. MS: metabolic syndrome, MS + TMS: metabolic syndrome + telmisartan 8 mg/kg, MS + ELE50: metabolic syndrome + *E.uniflora* leaf extract 50 mg/kg; MS + ELE100: metabolic syndrome + *E.uniflora* leaf extract 100 mg/kg. *p<0.05, **p<0.01 compared with MS (ANOVA and post-hoc test)

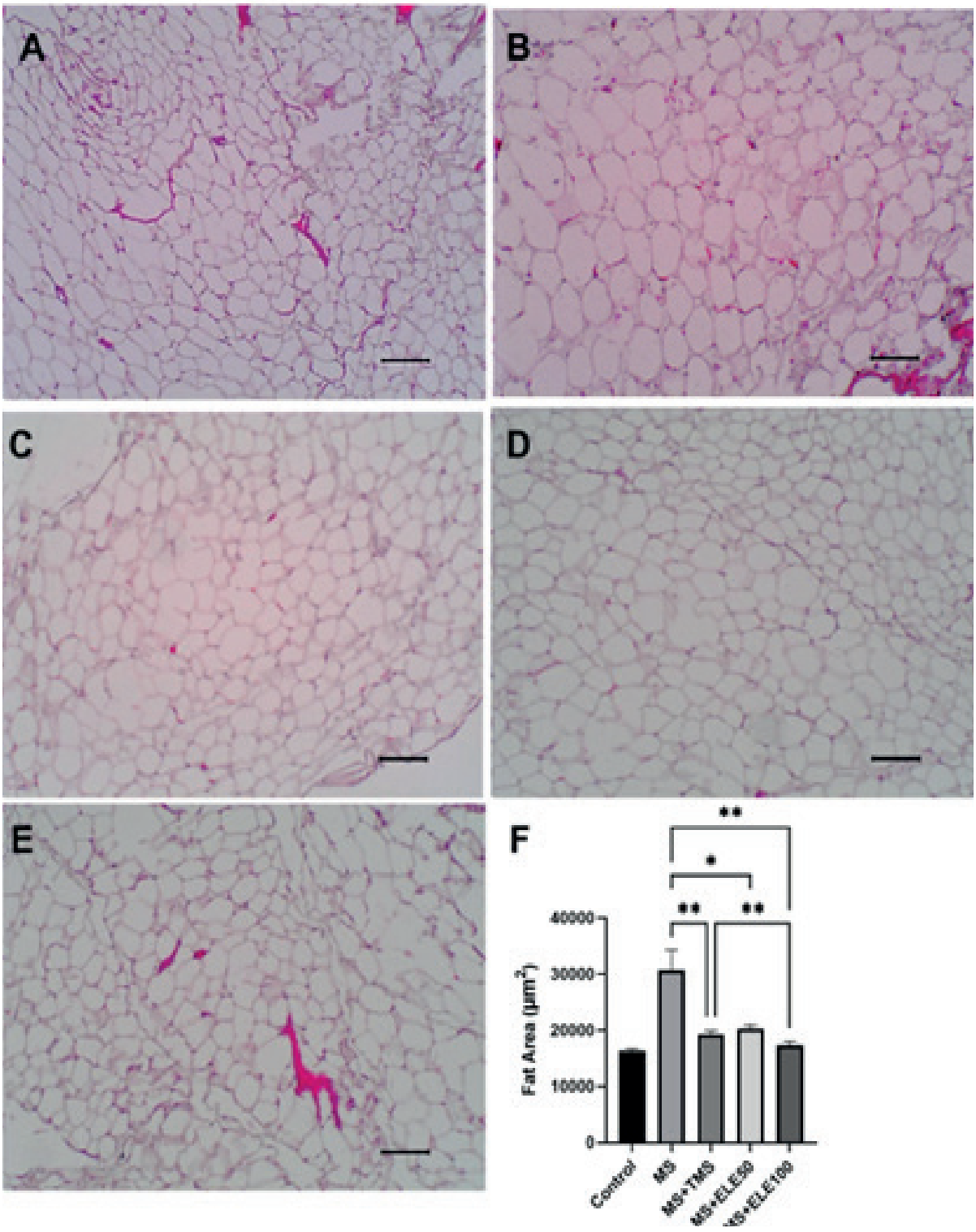
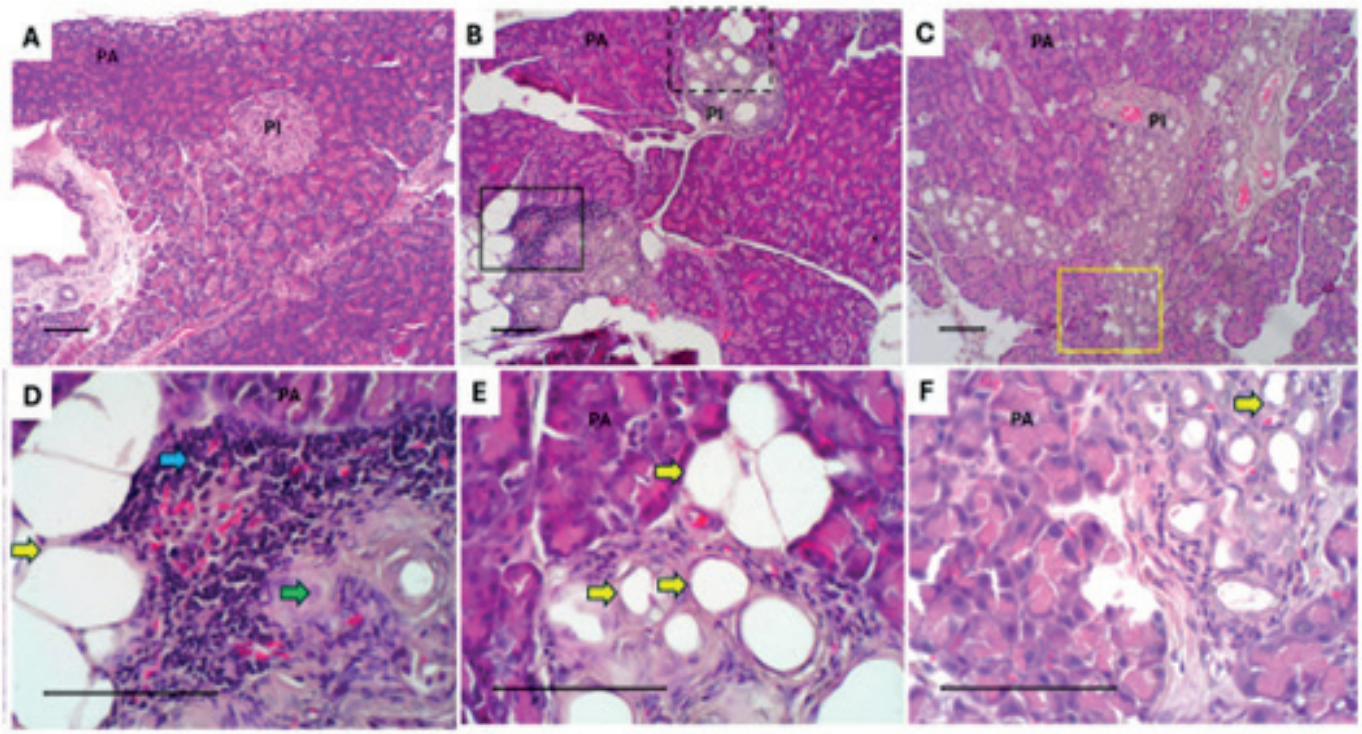


Figure 3. Effect of *E.uniflora* leaf extract on the fat area. A. Control, B. MS: metabolic syndrome. C. MS + TMS: metabolic syndrome + telmisartan 8 mg/kg, D. MS + ELE50: metabolic syndrome + *E.uniflora* leaf extract 50 mg/kg; E. MS + ELE100: metabolic syndrome + *E.uniflora* leaf extract 100 mg/kg. *p<0.05, **p<0.01 compared with MS (ANOVA and post-hoc test). Bar: 100 μm.



Group	Acinar Vacuolization	Insular Vacuolization	Inflammation and fibrosis
Control	0	0	0
MS	++	++	++
MS + TMS	0	+	0
MS + ELE50	+	++	0
MS + ELE100	0	+	0

Figure 4. Histopathological changes on the pancreas. Upper panel showed normal pancreas from control (A) and abnormal pancreas from MS (B) and MS+ELE50 (C), HE staining, bar: 100 µm; 100X. Continuous line box area was enlarged in D showing large adipocytes (yellow arrow) and lymphocytes infiltration (blue arrow) as well as fibrotic area (green arrow). Dashed line box area was enlarged in E showing vacuolization and adipocytes (yellow arrows). Insular vacuolization also observed at MS + ELE50 (C and F), 400X. Lower panel: recapitulation of qualitative pathological changes groups. MS: metabolic syndrome; MS + ELE50: metabolic syndrome + *E.uniflora* leaf extract 50 mg/kg. PA: pancreatic acini, PI: pancreatic islet.

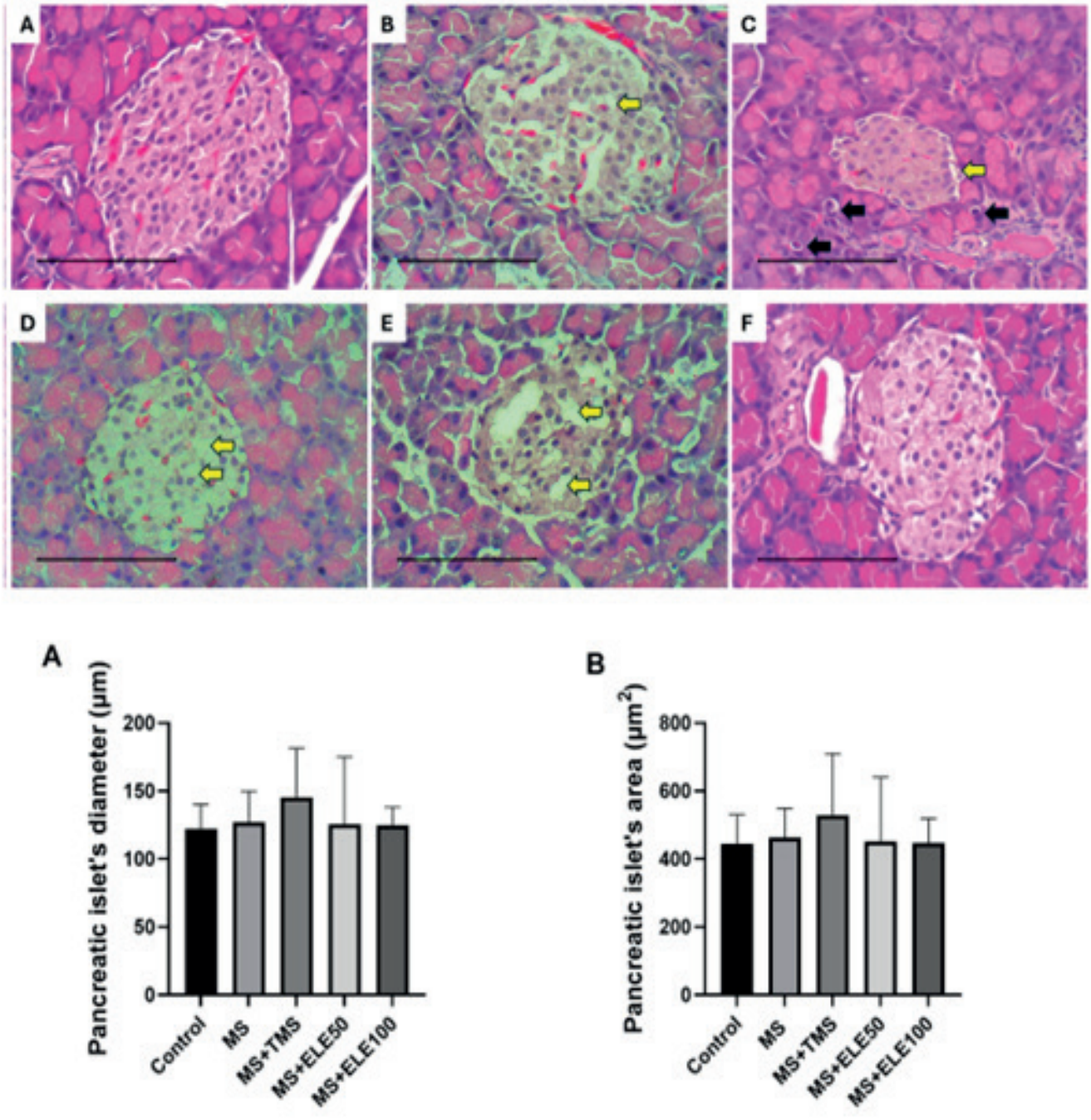


Figure 5. High magnification of pancreatic islets among groups. Upper panel showed normal islet in Control group (A), acinar vacuolization (black arrow) and insular vacuolization (yellow arrow) were observed in MS group (B and C). Insular vacuolization also observed at MS + TMS and MS + ELE50 (D and E) but markly reduced in MS + ELE100 (F). Lower panel: There were no difference in diameter (A) and area (B) of the Langerhans islets among groups. MS: metabolic syndrome; MS + TMS: metabolic syndrome + telmisartan 8 mg/kg; MS + ELE50: metabolic syndrome + *E.uniflora* leaf extract 50 mg/kg; MS + ELE100: metabolic syndrome + *E.uniflora* leaf extract 100 mg/kg HE staining, bar: 100 µm, 400X.

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