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Exploring the therapeutic potential of two different concentrations of chamomile (*Matricaria chamomilla* L.) root extracts for the management of Type-II diabetes mellitus

ABSTRACT

The study aims to determine the effect of chamomile (*Matricaria chamomilla L.*) root extracts for the management of type-II diabetes mellitus. In this experimental study fresh *Matricaria recutita L.* roots were harvested, cleaned, and crushed with 5ml liquid nitrogen. The crushed roots were dried and extraction was done by ultrasonic microwave extraction using 0.1 grams of roots with 10 ml 70% ethanol. Durations for extraction vary within of 5, 10, and 15 minutes. The liquid extract was converted it into powder form by spray drying.

The experimental rats groups were divided into five groups that include a control group, Diabetic rat group, diabetic rats provided with metformin as positive control group, and experimental group treated with dose of 100 mg/kg of chamomile root extract and other with 200 mg/kg of chamomile root extract. The mean values of total phenolic contents, DPPH activity, TFC activity, ABTS+ activity and FRAP activity was obtained high for the ultrasonic extraction done for 15 minutes with mean values of 23.95±1.22, 2.61±0.19, 4.06±0.23 and 31.56±2.04 respectively. Similarly, the level of Alanine Aminotransferase, Aspartate Aminotransferase was high for the group treated with high dose of chamomile (*Matricaria chamomilla L.*) root extracts. Overall, the level of Creatinine, Glucose, Insulin, Superoxide Dismutase, HbA1c l and GPx were also high for the rats treated with high dose of chamomile (*Matricaria chamomilla L.*) root extracts. The findings of this study showed important implications for the development of natural supplements for type-II diabetes mellitus management, which may be more accessible, cost-effective, and have fewer side effects than pharmaceutical interventions.

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Keywords: Chamomile (*Matricaria chamomilla L.*) Root, Type-II Diabetes Mellitus, Antioxidant.

1. INTRODUCTION

1.1. Historical perspective:

Diabetes mellitus (DM) is a collection of metabolic disorders that have different origins, characterized by increased levels of glucose in the bloodstream or chronic hyperglycemia, as well as insulin secretion abnormalities, insulin resistance, or a combination of both. It is considered to be one of the most significant health hazards facing people worldwide today (Al-Sowayan & AL-Sallali, 2023). Type-II Diabetes Mellitus, which is characterised by insufficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR), and an insufficient compensatory insulin secretory response, accounts for about 90% of all instances of diabetes mellitus (Galicia-Garcia et al., 2020). The impact of diabetes mellitus (DM) on global health is a cause for concern. According to the latest report by the International Diabetes Federation, it is estimated that by 2045, approximately 783 million individuals worldwide will be affected by DM, with type 2 diabetes mellitus (T2DM). This projection highlights the urgent need for effective prevention and management strategies to curb the rising prevalence of DM and its associated complications (Magliano et al., 2021). The commonness of type 2 diabetes (T2D) has been on the rise in Pakistan in recent decades, and this trend has significant implications for public health. Research directed by Basit and colleagues indicates that the prevalence of T2D in Pakistan is alarmingly high, with estimates of 26.3% in urban areas and 17.7% in rural areas. These findings underscore the need for increased awareness and effective interventions to address this growing health concern in the country (Basit et al., 2018). Initially, in the early stages of type II diabetes mellitus, there may be only a minor elevation in blood sugar levels. However, if left uncontrolled, the disease can progress and give rise to severe complications, including diabetic foot ulcers, diabetic kidney damage, and diabetic retinopathy (Cole & Florez, 2020). These complications can cause significant pain and discomfort for the patient, as well as impose a substantial economic burden. It is, therefore, crucial to diagnose and manage T2DM effectively to delay or prevent the beginning of these complications and improve the quality of life for those living with the disease (Harding et al., 2019; Zemestani et al., 2016). Scientists have made significant progress in developing various medications, such as

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insulin and metformin, that reduce blood sugar levels in persons with type 2 diabetes by using several processes. However, despite these advances, individuals with T2DM often encounter difficulties in controlling their blood glucose levels effectively(Guo et al., 2021). These challenges include experiencing high fluctuations in blood glucose levels, being at risk of hypoglycemia, and facing a high incidence of adverse gastrointestinal reactions to certain medications. Some antihyperglycemic drugs, such as metformin, may cause gastrointestinal issues. Sulfonylureas can lead to hypoglycemia and weight gain. Insulin use is associated with the risk of hypoglycemia and weight gain. Addressing these issues is crucial in managing T2DM effectively and improving outcomes for patients. Given the difficulties patients have controlling their blood sugar levels when they have type 2 diabetes mellitus (T2DM) effectively, it is imperative to explore the development of safe and effective adjunctive therapies for this condition (White Jr, 2014). Such treatments could potentially complement the existing medications and help patients achieve better glycemic control, thereby reducing the risk of complications associated with T2DM. For millennia, traditional medicine has used herbs to treat and prevent a variety of illnesses. They are known for their remarkable efficacy and minimal side effects, which has led to their widespread use in many cultures across the world (Huang et al., 2018). Chamomile (*Matricaria chamomilla* L.) is a valuable medicinal herb that is indigenous to Europe and Asia. It belongs to the Asteraceae family, which was formerly known as Compositae. Chamomile has been utilized for medicinal purposes in traditional medicine for an extended period (Mežaka et al., 2020). It is cultivated in various regions across the globe. It is mostly cultivated in western North America, southern and eastern Europe, central and western Asia and northern Africa (Chauhan et al., 2022). The term "chamomile" has its roots in ancient Greece and is a combination of two Greek words, "Chamos" and "Melos," which translate to "ground apple." This name is a reference to the herb's characteristic apple-like fragrance (El Mihyaoui et al., 2022). There are various botanical species commonly referred to as chamomile. German chamomile (*Matricaria chamomilla* L.) is perhaps the most popular species and is widely used in traditional medicine and herbal remedies. Other species of chamomile include wild chamomile (*Matricaria discoidea* DC.), scentless or false chamomile (*Tripleurospermum inodorum* L.), stinking chamomile (*Anthemis*

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cotula L.), Matricaria aurea Loefl., field or corn chamomile (*Anthemis arvensis* L.), Valley mayweed (*Matricaria occidentalis* G.), dyer's chamomile (*Cota tinctoria* L.), among others. Each species has its unique properties and potential health benefits (Tsivelika et al., 2021). To avoid confusion, it is now widely accepted that *Matricaria recutita* L. (also known as Chamomilla recutita) is the scientific name for chamomile, which is a member of the Asteraceae family of the genus Chamomilla L. One of the distinguishing features between true chamomile (*Matricaria recutita* L.) and other chamomile varieties is that the other species of chamomile may have an odorless smell or a pungent, stinking odor (Franz et al., 2007). Chamomile is frequently used as a tea or tonic and is usually regarded as safe for eating. It has been utilized for millennia in conventional medical practices, such as Unani and homoeopathy, for its various therapeutic properties (Shareef et al., 2016). In 2000, the use of chamomile as an active component in over-the-counter (OTC) dietary supplements was authorized by the US Food and Drug Administration (FDA). This decision was based on the chamomile plant's effectiveness and safety as well as it has been utilized for medicinal purposes in traditional medicine for an extended period. Additionally, German chamomile is classified as GRAS (generally recognized as safe) for use in food products by the FDA. The USFDA has also recognized essential oil, extracts, and distillates of chamomile as generally regarded as safe (GRAS). This demonstrates the crucial part in food industry that chamomile is playing. Nutraceuticals, which are food products that offer health benefits beyond basic nutrition, have gained importance in disease prevention. If the chamomile active component is discovered and researched in a method that provides high bioavailability and effectiveness, it can be explored for use as a nutraceutical agent. Chamomile has been included as a drug in the pharmacopoeia of 26 countries, which further highlights its importance as a medicinal herb (Al-Dabbagh et al., 2019).

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Chamomile is indeed a popular herb that has been used for centuries for various medicinal purposes, including as an herbal tea. The extract of chamomile has been found to have several potential health benefits, including pain relief, antiseptic, antibacterial, antioxidant, and anti-inflammatory activity. It is believed that chamomile extract functions as analgesic. Additionally, it possesses antioxidant, antibacterial, antiseptic and anti-inflammatory properties as well as Type 2 diabetes,

also defence against non-alcoholic fatty liver disease and some forms of cancer. The health and medicinal benefits of chamomile are believed to be attributed to its bioactive components, which are primarily found in the essential oil and aqueous extract. The essential oil is rich in sesquiterpenes such as chamazulene,α-bisabolol, and bisabolol oxides A and B, while the aqueous extract contains mostly phenolic acids, flavonoids, and coumarins (Tsivelika et al., 2021). The principal phenolic substances present in chamomile are reported to be herniarin and umbelliferone, which are classified as coumarins. In addition, chamomile contains caffeic acid and chlorogenic acid, which belong to the phenylpropanoid group of compounds. Flavones such as apigenin, luteolin, luteolin-7-O-glucoside and apigenin-7-O-glucoside, as well as flavonols such as rutin and quercetin, are also identified as the primary phenolic compounds found in chamomile (Dikeman & Devine, 2014). The chamomile roots included a significant quantity of polyphenolic compounds and flavonoids, with the main ingredients being luteolin O-acylhexoside, catechol, ellagic acid, querctin and chlorogenic acid) at the following concentrations: 2801.99, 1104.49, 1582.81, 1765.01and 937.48 (A Elsemelawy, 2017).Chamomile decoctions and extracts typically contain flavonoids, such as apigenin, in the form of glycosides. These glycosides are highly stable and water-soluble, making them readily available for extraction and consumption (Pereira et al., 2018).

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In traditional Moroccan medicine, the flowers of M. chamomilla (known locally as Babonj/Babounj) are commonly used for medicinal purposes, followed by the leaves and the whole plant. The plant parts are typically prepared as an infusion or decoction and are used to treat diabetes (Mrabti et al., 2019; Naceiri Mrabti et al., 2021).

1.2. Limitations of the study:

- Limited generalizability to humans: Animal studies may not always be directly applicable to humans due to biological differences between species.
- Potential for extrapolation errors: Findings from animal studies may not always be accurately extrapolated to humans, which could lead to errors in clinical decision-making.
- Ethical concerns: There may be ethical concerns regarding the use of animals in research, which could limit the ability to conduct certain types of studies.

- Environmental factors: Animal studies may not fully capture the complex environmental factors that may impact disease progression or treatment outcomes in humans.
- Potential for experimental artifacts: Experimental artifacts, such as differences in animal handling or diet, could impact study outcomes and introduce bias into the results.

1.3. Problem statement:

Type-II diabetes mellitus is a prevalent chronic disease worldwide, and despite advancements in medication, its management is still a challenge. The impact of diabetes mellitus (DM) on global health is a cause for concern. According to the latest report by the International Diabetes Federation, it is estimated that by 2045, approximately 783 million individuals worldwide will be affected by DM (Magliano et al., 2021). The commonness of type 2 diabetes (T2D) has been on the rise in Pakistan in recent decades. Research directed by Basit et al. (2018) indicates that the prevalence of T2D in Pakistan is alarmingly high, with estimates of 26.3% in urban areas and 17.7% in rural areas. In Furthermore, the side effects and limitations of current medications make alternative therapies an attractive option due to the side effects associated with medicines. Chamomile (*Matricaria chamomilla* L.) is a natural plant extract that has been traditionally used for medicinal purposes, including the management of diabetes. The chamomile roots included a significant quantity of polyphenolic compounds and flavonoids, with the main ingredients being luteolin O-acylhexoside, catechol, ellagic acid, querctin and chlorogenic acid) at the following concentrations: 2801.99, 1104.49, 1582.81, 1765.01and 937.48 (A Elsemelawy, 2017). However, the optimal concentration of chamomile root extract for the management of Type-II diabetes mellitus remains unclear. Since the majority of plant components used for medicine are above-ground, roots have thus far received relatively little attention. Therefore, the problem statement is to investigate the therapeutic effects of two different concentrations of chamomile root extract in diabetic rats and to determine the optimal concentration of chamomile root extract for the management of Type-II diabetes mellitus.

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1.4. Hypothesis:

Null hypothesis (H_0):

1. There is no significant difference in blood glucose levels between diabetic rats that are given chamomile root extracts and those that are not given chamomile root extracts.
2. Diabetic rats that are given chamomile root extracts will not have significant effect on insulin sensitivity compared to the rats that are not given chamomile root extracts.
3. There is no significant effect of chamomile root extracts on safety parameters.

Alternative hypothesis (H_1):

1. Diabetic rats that are given chamomile root extracts will have significantly lower blood glucose levels compared to diabetic rats that are not given chamomile root extracts.
2. Diabetic rats that are given chamomile root extracts will have significant effect on insulin sensitivity compared to the rats that are not given chamomile root extracts.
3. There is significant effect of chamomile root extracts on safety parameters.

1.5. Objectives:

1. To investigate the therapeutic potential of two different concentration of chamomile root extracts on blood glucose levels of diabetic rats.
2. To investigate the therapeutic outcomes of two different concentration of chamomile root extracts on insulin sensitivity of diabetic rats.
3. To evaluate the outcomes of two different concentration of chamomile root extracts on safety parameters of diabetic rats.

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2. LITERATURE SURVEY

Murti and colleagues. carried out research to comprehend the potential anti-diabetic effects of *Matricaria recutita* roots on rats that had been induced with Streptozotocin (STZ). The researchers administered an ethanolic extract of the roots to the rats and used glibenclamide as a standard drug. The study conducted by Murti et al. involved measuring blood glucose levels at different time points following oral ingestion of *Matricaria recutita* ethanolic extracts (the dose administered was 400 mg/kg.). Four distinct days were used to assess the blood sugar levels: day 0 (before the extract was administered), day 7, day 14, and day 21 after the extract was administered. The outcomes demonstrated that the ethanolic chamomile extract might reduce blood sugar levels in the STZ-induced diabetic mice, with a significant drop observed from the 7th day of continuous administration of the extract. These findings suggest that *Matricaria recutita* may possess glucose-lowering effects (Murti et al., 2011).

This study aimed to investigate the effects of chamomile flower and root extracts on diabetic rats and analyze the content of polyphenolic compounds and flavonoids in chamomile. The results revealed that the extracts contained various polyphenolic compounds and flavonoids. Diabetic rats exhibited adverse changes in various parameters compared to healthy rats. However, treatment with 10% and 20% chamomile extracts resulted in improvements in feed intake, HDL-C, acetylcholine esterase, catalase activity, and glutathione activity. Conversely, other parameters showed significant decreases compared to the positive control group. The study concludes that chamomile extract improved the nutritional and biochemical status of diabetic rats, particularly at a 20% concentration (A Elsemelawy, 2017).

The effects were examined in research of chamomile extract supplementation, physical exercise, and their combination on diabetic rats were investigated. For a period of eight weeks, the rats were administered chamomile extract through gavage at a dosage of 200 mg/kg, subjected to physical exercise, or given a combination of both treatments. The findings indicated that the two were combined of chamomile extract supplementation and physical exercise, fasting plasma glucose (FPG) decreased by 53% and insulin levels increased by 61% compared to diabetic controls. The individual treatments of chamomile extract supplementation and physical

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exercise also showed significant increases in insulin levels and decreases in FPG in comparison with to diabetic controls. These verdicts suggest that the combination of chamomile extract supplementation and physical exercise may be an effective approach for managing diabetes by improving insulin sensitivity and glycemic control (Amir et al., 2017).

In research conducted, mice with induced diabetes with alloxan were given an aqueous solution chamomile (leaves, stems, flowers, or all aerial parts) for a period of six weeks. There were two different dosages used: 150 and 300 mg per kg of the body weight. The study's findings showed that the combination of low dosages of both extracts and higher doses of the extracts significantly increased serum insulin levels and HbA1c and decreased amylase activity. Aside from that the group that received 150 mg per kg chamomile extract demonstrated a reduction of 20% in fasting plasma glucose (FPG) levels, while group given 300 mg per kg of chamomile extract experienced a reduction of up to 57% in FPG levels (Prasanna et al., 2017).

A research was conducted, oral extract of chamomile flowers at a dosage of 500 mg/kg using feeding cannels to STZ-induced diabetic rats in their study. After four weeks, the research showed a notable decrease of up to 64% in serum glucose levels, but no changes were observed in insulin levels (Al-Musa & Al-Hashem, 2014).

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Researchers carried out research to investigate the potential effect of chamomile flower extract on diabetic male rats. The rats were given a dosage every day of 200 mg/kg extract in their drinking water, with a daily dose of 200 mg/kg. The administration period was 14 weeks. The results showed that the rats who received the extract had increased levels of insulin and improved insulin sensitivity. Additionally, they showed a decrease in glucose intolerance, as evidenced by lowering in fasting blood sugar levels of up to 19% and a decrease in serum GPLD1(Glycosylphosphatidylinositol specific phospholipase D) levels. However, the levels of glypican-4 in the supplemented group remained unchanged when contrasted with the control group. These results indicate that chamomile flower extract may have beneficial effects for individuals with diabetes (Abdolmaleki & Heidarianpour, 2018).

Researchers investigated effects of chamomile leaf extract on diabetic rats induced with STZ in a research. Through a stomach cannula, 200 mg/kg of chamomile leaf extract was administered to the rats for three weeks. According to the study, rats given

chamomile leaf extract exhibited a substantial 52% decrease in blood glucose levels when in comparison to the control group. The researcher also observed that the rats treated with chamomile leaf extract had improved glucose metabolism and insulin sensitivity. This suggests that chamomile leaf extract may regulate blood sugar levels and enhance insulin sensitivity in people with diabetes. The findings of the study imply that chamomile leaf extract may serve as a natural treatment option for diabetes (Emam, 2012).

Rafraf and colleagues conducted a research experiment that is randomized control trials and hypoglycemic to examine the impact of chamomile tea drinking on people with type 2 diabetes. The study included 64 participants who were randomly allocated to either a chamomile tea group or a control group. Participants in the chamomile tea group consumed 3 g of chamomile tea in 150 mL of hot water three times per day for eight weeks. As stated by the study's findings, consumption of chamomile tea resulting to a substantial drop in levels of glycosylated hemoglobin (HbA1C), serum insulin and insulin resistance in comparison to the control group. Glycosylated hemoglobin (HbA1C) is a marker of blood glucose levels over a period of 2-3 months, and its reduction indicates an improvement in the long-term management of blood sugar. Serum insulin levels and insulin resistance are also important indicators of glucose metabolism, and their decrease suggests an improvement in glucose homeostasis. Chamomile has been suggested to have anti-inflammatory and antioxidant properties, which may contribute to its hypoglycemic effects. It has also been shown to stimulate insulin secretion and enhance glucose uptake in peripheral tissues. The study's findings generally indicate that chamomile tea consumption may have beneficial effects for patients with type 2 diabetes by increasing insulin sensitivity and glucose regulation (Rafraf et al., 2015).

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Researchers conducted a research study with the aim of investigating the potential therapeutic effects of chamomile tea in a rat model of diabetes caused by STZ. The researchers supplemented the rats with chamomile tea to report the effects of chamomile methanolic extract by giving the rats a quantity of 500 mg/kg over the course of 21 days, the effects on plasma glucose levels and hepatic glycogen phosphorylase activity were assessed. According to the findings, plasma glucose levels were significantly reduced by up to 14%. This drop in blood sugar levels was

attributed to the inhibitory effects of chamomile tea on hepatic glycogen phosphorylase, this is an enzyme responsible for the liver's synthesis of glucose from glycogen. The results of this investigation indicate that chamomile tea may have anti-diabetic effects y helping to regulate blood sugar levels (Kato et al., 2008).

Cemek and colleagues carried out a research to investigate the effect of different doses of chamomile extract on diabetic rats caused by STZ. The rats received chamomile extract treatment (aerial parts) by gavage for 14 days at dosages of 20, 50, and 100 mg/kg body weight. The researchers evaluated the extract's effects on insulin-positive β-cells of pancreatic islets and serum glucose levels. The study's findings revealed that chamomile extract increased the number of insulin-positive β-cells in pancreatic islets compared to a dose-dependent manner to the non-treated animals. Additionally, the extract significantly decreased high levels of serum glucose in a way dependent on dosage, with reductions of 5%, 15%, and 23% in the chamomile 20, chamomile 50, and chamomile 100 groups, respectively. These findings suggest that chamomile extract may have potential to treat diabetes naturally by increasing the number of insulin-positive beta cells and lowering high blood glucose levels (Cemek et al., 2008).

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The study, examined the impact of chamomile extract on postprandial blood glucose levels in diabetic rats induced by STZ. The effects of a single dosage and daily oral administration of an aqueous extract of chamomile's aerial parts (leaf/flower) on blood sugar levels were studied by the researchers. The chamomile extract was administered to the rats either as a single oral dose of 20 mg/kg or as a daily oral treatment at the same concentration for 15 days. The researchers revealed that daily administration of chamomile extract results in rats with STZ-induced diabetes seeing a substantial 61% drop in postprandial blood glucose levels. Additionally, they saw a 4% drop rate in blood glucose levels following a single oral dosage of the extract. However, the variations in insulin plasma levels weren't statistically significant. According to these findings, chamomile extract has potential as a therapeutic agent in the management of diabetes, as it can notably lower postprandial blood sugar levels. The research also indicates that the effects of chamomile extract are more pronounced with daily oral administration compared to a single dose. The findings suggest that chamomile extract shows promise as a natural

treatment option for diabetes by potentially lowering postprandial blood sugar levels (Eddouks et al., 2005).

The scientists conducted a study to look at the impact of chamomile flower tea on alloxan-induced diabetic rats. The rats were given 1 g of chamomile flower tea for a duration of 8 weeks. According to the research, there was a notable decline in HbA1c levels as well as post-prandial and fasting blood glucose levels. The decrease in HbA1c levels was up to 59%, while the decrease in blood glucose levels up to 41% higher while fasting and post-prandial compared to baseline. The study suggests that chamomile flower tea may have a therapeutic effect on diabetes management by improving glucose control. The reduction in HbA1c levels and blood glucose concentrations indicates that chamomile flower tea may have potential as a natural remedy for diabetes. In conclusion, Khan.'s study shows that chamomile flower tea may be a promising natural treatment for diabetes by improving glucose control. The study's results suggest that chamomile flower tea may be a useful addition to conventional diabetes management strategies, but more research is necessary to confirm its effectiveness and safety in the long term (Khan et al., 2014).

In their study, scientist aimed to assess the outcome chamomile tea on blood glucose levels during fasting and post prandial. Participants consumed chamomile tea twice daily for four weeks, with each cup containing 10g of chamomile steeped in 100ml of boiling water. The study found that the consumption of chamomile tea resulted in a significant reduction in HbA1c values, as well as fasting and post-prandial blood sugar concentrations. Post-prandial blood glucose levels decreased by up to 7%, while fasting blood glucose levels dropped by up to 8% compared to baseline. The study suggests that chamomile tea may have a beneficial influence on diabetes patients' ability to manage their blood sugar. The study's findings indicate that chamomile tea may be a promising natural remedy for the management of diabetes by improving blood glucose control. The chamomile tea consumption may help improve blood glucose control in individuals with diabetes. Chamomile tea may be a beneficial addition to conventional diabetes management strategies, but more research is necessary to confirm its effectiveness and safety in the long term (Kaseb et al., 2018).

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Zemestani and colleagues conducted a clinical trial with two groups of 64 individuals with type 2 diabetes mellitus (T2DM) were randomly allocated to each other. For eight weeks, one group got chamomile tea (3 g/150 ml hot water), and the other received a placebo. The results of the trial revealed that the consumption of chamomile tea significantly reduced insulin resistance, blood insulin levels, and HbA1c concentrations. among the participants, compared to those who received the placebo. Moreover, the serum glucose levels in the chamomile tea group were also remarkably reduced by up to 11%, although there were no significant changes in the between-group comparison or in the placebo group (Zemestani et al., 2016).

A study conducted by Kermanian and fellows examined the outcome of chamomile tea on depression and glycemic control in subjects with diabetes. 64 participants were involved in a study where they were randomly assigned to receive either black tea or chamomile tea. They consumed three cups of the assigned tea per day for a duration of eight weeks. The results showed that the consumption of chamomile tea significantly improved depression scores compared to the black tea group. Additionally, the chamomile tea group HbA1c values were significantly lower in the green tea group than in the black tea group. The results of the study imply that chamomile tea may benefit diabetes patients' emotional and physical wellbeing (Kermanian et al., 2018).

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3. Materials and Methods

3.1. Proposed place of work and facilities available:

The research was conducted in the research laboratory at the University Institute of Diet and Nutritional Sciences, The University of Lahore.

3.2. Plan of work and methodology adopted:

1. Procurement

Materials

The roots of Chamomile (*Matricaria recutita* L.*)* will be collected from local market of Lahore.

Sample preparation of the root extract

To explore the therapeutic potential of chamomile root extracts for managing Type-II Diabetes Mellitus, fresh Matricaria recutita L. roots were harvested, cleaned, and crushed with 5ml liquid nitrogen. The crushed roots were dried at 45°C and stored. Extraction was done using 0.1 grams of roots with 10 ml 70% ethanol in a 125 ml flask, followed by filtration. Ultrasonic microwave extraction was conducted with parameters ranging from 0 to 700 W intensity and durations of 5, 10, and 15 minutes (Patel et al., 2018; Abdelfatah et al., 2019; Morsy et al., 2017). Standardized parameters in the ultrasonic microwave system included agitation at 400 rpm, a temperature of 45°C, and a plant-to-solvent ratio of 1:20. The liquid extract obtained were undergone spray drying to convert it into powder form, preserving its active compounds. The powder offers enhanced stability, ease of handling, and prolonged shelf life, suitable for further analysis and storage. The prepared powder was utilized in overall experiment (Laina. K et al., 2021).

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Extract Analysis

For the analysis of the extract of chamomile root and to determine its antioxidant properties following test were performed which include:

- TPC (Total Phenolic Content)
- TFC (Total Flavonoid Content)
- 1,1-diphenyl-2-picrylhydrazyl (DPPH)
- 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay

- Ferric reducing antioxidant power (FRAP) assay

Total phenolic content

With some changes, the Folin Ciocalteu's reagent developed by Singleton and Rossi (Singleton & Rossi, 1965) was used to quantify the total amount of phenolic compounds present in the samples. To perform this test, 50 microliters of sample (hydrophilic or lipophilic phase) was added to a glass test tube, followed by the addition of 950 µl of distilled water, 50 µl of 1 M sodium carbonate, and 50 µl of Folin Ciocalteu reagent. The combination was then allowed to sit for 15 minutes at 30°C in a water bath before the absorbance is gauged at 715 nm. Gallic acid was utilized as a reference to estimate the content of phenolic compounds in the sample. On a Perkin Elmer Lambda-2S UV-VIS spectrophotometer, photometric measurements were taken and the findings were presented as mg gallic acid equivalents per gramme of dry weight (mg GAE g^{-1} DW). To ensure accuracy, experiments was conducted in triplicates, and mean and standard deviation values were calculated (EL MIHYAOUI et al., 2021).

Total flavonoids content

The research apply Woisky and Salatino's modified aluminium chloride colorimetric technique to ascertain the total flavonoid content in the plant samples (Woisky & Salatino, 1998). The standard was quercetin, which was further dissolved in 80% ethanol and diluted to 25, 50, 100, and 150 µg. mL^{-1}. Then, 2.8 ml of distilled water was added along with 1.5 ml of 95% ethanol, 0.1 ml of 1 M potassium acetate, 0.1 ml of 10% aluminium chloride, and 0.5 ml of the diluted standard solutions. The 30-minute room temperature incubation was provided followed by a 415 nm absorbance measurement of the reaction mixture. For the determination of content of flavonoids in the plant samples, the same approach was used. The outcomes were presented in milligrammes of quercetin equivalents per gramme of dry weight (mg QE·g^{-1} DW). The experiments was conducted in triplicates to ensure accuracy, and means and standard deviation values were calculated (EL MIHYAOUI et al., 2021).

DPPH radical-scavenging assay

To determine the DPPH radical scavenging activity, the mixture of antioxidants were added to 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol

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solution, and the steady-state absorbance was measured using the method of Liyana-Pathiranan and Shahidi (Liyana-Pathirana & Shahidi, 2005). A final concentration of 4 mg/mL of the extract was obtained by dissolving it in 10 mL of pure ethanol. Then, 2 mL of 0.004% (0.2 mM) DPPH in ethanol was mixed with 1 mL of the extract solution. To measure the absorbance at 517 nm, the mixture was forcefully mixed and immediately put in a UNICO UV-2100 spectrophotometer until it reaches a plateau. Ascorbic acid was used as a reference standard, a stable antioxidant. The DPPH radical-scavenging activity was then calculated using the following equation in percentage of sample (Yen & Duh, 1994):

Inhibition percentage $(I_p)= 100(A_B - A_A)/A_B$

where A_B and A_A represent the absorbance values of the blank and test samples, respectively, measured after 70 min. All the data with means (± standard deviations) of triplicate determinations of three independent tests was calculated (Zhang et al., 2013).

Determination of ABTS$^+$ 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)

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The spectrophotometric measurement of ABTS+ scavenging activity was conducted using the procedure outlined by Rufino et al. (Rufino et al., 2007). 7 mM ABTS and 140 mM potassium persulfate were combined to create the ABTS+ cation radical, which was then be kept at room temperature for 16 hours in the dark. The ABTS+ solution was diluted before use in a spectrophotometer until an absorbance of 0.950 ± 0.050 at 734 nm is achieved. Then, 10 mL of methanol was added to 1 mL of ABTS+ solution at various concentrations [(1:1), (3:4), (1:2), and (1:4) (v/v)]. The percentage of inhibition at 734 nm was estimated after 6 minutes of each concentration by comparing the sample's absorbance to that of methanol. Trolox was used as a standard reference (a synthetic vitamin E analogue). The equation derived from the common trolox curve was utilized to determine the scavenging capacity of ABTS+ radicals (at a trolox range of 500–2000 μM). The results are given in μM trolox/g extract. The tests was performed in triplicates (Gerolis et al., 2017).

Determination of FRAP (ferric reducing/antioxidant power)

With very minor adjustments, the FRAP test (Benzie & Strain, 1996) was employed. Acetate buffer (300 mM, pH 3.6), 10 ml of 1,3,5-tri(2-pyridyl)-2,4,6-triazine solution in HCl 40 mM, and 20 mM iron (III) chloride solution was mixed in the following ratios: 10:1:1 (v/v/v), respectively, to prepare the FRAP reagent. The resulting mixture before use was warmed to 37°C in a water bath. To carry out the assay, 100 µL of calibration solution, was added to 3 mL of the FRAP reagent. The identical test tubes contain the standard, which was incubated at 37°C for 5 minutes. The FRAP working solution was used as the blank to measure the absorbance at 593 nm. Based on the sample's capacity to eliminate ferric ions, its antioxidant capacity was determined and reported as mmol FeSO4 equivalents per gramme of sample (DW), using the linear calibration curve (Zhang et al., 2013).

Animals

The study utilized 20 male Wistar rats that weight in between 180 and 200 g at 6 to 8 weeks of age. The rats were provided with standard laboratory pellet rodent diet and unlimited access of water. The rats were kept in a controlled environment at a temperature of 21.8°C and humidity of 60%, with 12 hours of light and 12 hours of darkness. The allocation of rats to cages was done randomly, with a maximum of five rats per cage.

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Induction of diabetes

A freshly made solution of streptozotocin (STZ) at the dosage of 60 mg/kg of body weight in 0.1 M citrate buffer, pH 4.5, was injected intraperitoneally to cause diabetes in the rats after an overnight fast in which the rats were denied food for 16 hours but were given free access to water. To prevent hypoglycemia shock-related mortality, rats treated with STZ were provided with a 5% glucose solution instead of water. After seventy-two hours, fasting blood glucose levels were measured using a One Touch glucometer. Those rats with fasting blood glucose levels exceeding 250 mg/dL were classified as diabetic rats. The treatment was commenced on the seventh day after the STZ injection, and this day was considered the first day of treatment (Al-Sowayan & AL-Sallali, 2023).

Standard Diet

The rats in the study were fed a standard diet that comprised a mixture of corn, full-fat soy derived from genetically modified soybeans, sunflower seed meal, wheat flour, wheat ram, alfalfa flour, beef meat-bone-chicken flour, sugar beet molasses, calcium carbonate, dicalcium phosphate (inorganic), vitamin and mineral premix. This diet was carefully formulated to provide a balanced combination of essential nutrients (BARCIN-GÜZELDERE et al., 2022).

2. Selection Criteria:

i. Inclusion Criteria:

- ✓ Male Wistar rats were included.
- ✓ Rats between 6-8 weeks of age were included.
- ✓ Rats weighing between 180-200 g were included.
- ✓ Fasting blood glucose levels <100 mg/dL were included.

ii. Exclusion criteria:

- ✓ Rats with other pre-existing medical condition were excluded.
- ✓ Rats previously enrolled in other studies were excluded.

3. Ethical considerations

The rules and regulations set by the ethical committee of university of Lahore were followed while conducting the research and the rights of the research participant's/research subjects were respected.

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Following considerations were strongly considered during animal trials:

- Animals were kept under controlled environment in clean, ventilated and properly designed cages.
- Proper diet and light & dark periods was maintained throughout the study.
- Any kind of harm (e.g., administration of banned chemicals etc.) to the animal was avoided.
- Environment provided would be close to the natural habitat of the specie in order to reduce stress.
- All information and data collection will be kept confidential.

3.3. **Treatments to be studied:**

As per the proposed study, the effects of two different concentrations of chamomile (*Matricaria chamomilla* L.) root extracts regarding the management of Type-II diabetes mellitus was studied. The treatment plan involved administering the

chamomile root extracts to the diabetic rats in treatment groups. Five of the rats were first divided at random and designated as the normal control group (-ve control); they received a conventional diet and 2 mL of water through gavage throughout the research, along with a placebo. Male Wistar rats having diabetes type 2 mellitus induce with streptozotocin was divided randomly into four groups. The diabetic control group was given no treatment. The positive control group (+ve control) was treated with metformin. The chamomile treatment group 1 received a standard diet, along with 100 mg/kg of chamomile root extract. The chamomile treatment group 2 received a standard diet and 200 mg/kg of chamomile root extract. The chamomile extract was administered orally for 21 days. The five groups comprised are following:

(i) Negative control group (-ve control) (n=5)- Healthy rats + Placebos

(ii) Diabetic control Group (n=5)- Diabetic rats

(iii) Positive control group (+ve control) (n=5)- Diabetic rats + metformin

(iv) Chamomile treatment Group 1 (T1) (n=5)- Diabetic rats +100 mg/kg of chamomile root extract

(v) Chamomile treatment Group 2 (T2) (n=5)- Diabetic rats + 200 mg/ kg chamomile extract.

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The study's objective was to assess the impact of chamomile root extracts on various parameters such as fasting blood glucose levels, insulin levels and hbA1c. The study also aimed to determine the optimal concentration of chamomile root extract for the management of Type-II diabetes mellitus. The treatment was compared to the three-control groups.

3.4. Research layout plan:

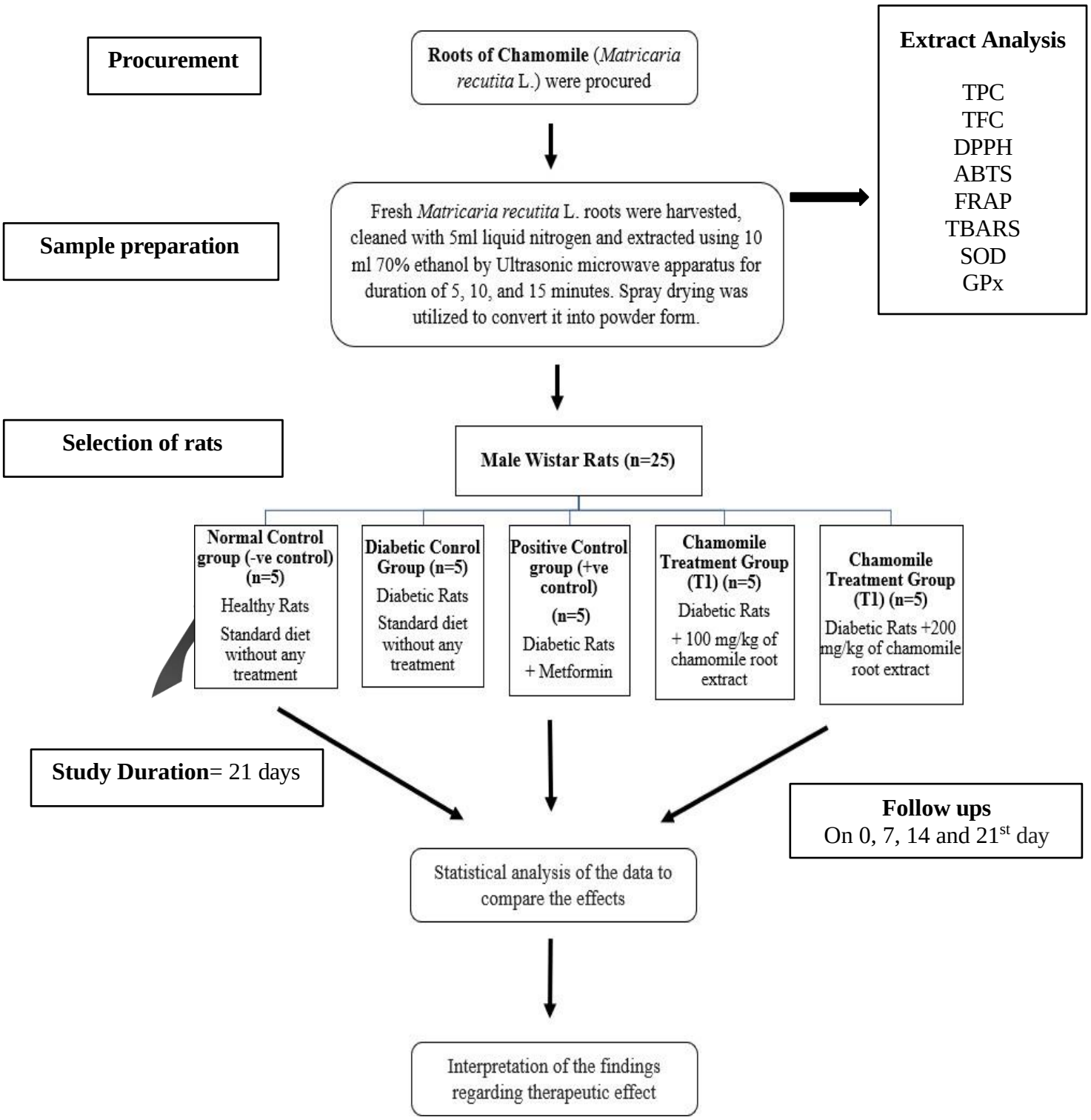

Figure 3.1 Research layout plan

3.5. Parameters/variables to be studied:

1. Blood glucose levels using a glucometer following the guidelines by American dietetic association guidelines (Association, 2014)
2. Serum levels of insulin by following the guidelines (Guarino et al., 2013)
3. Hemoglobin A1C (HbA1c) following the guidelines of American dietetic association guidelines (Association, 2014)
4. Liver Function Tests (LFTs) by following the guidelines (Thapa & Walia, 2007)
5. Renal Function Test (RFTs) by following the guidelines of (Gowda et al., 2010)
6. Total phenolic content (TPC) by following the guidelines of (El Mihyaoui et al., 2022)
7. Total flavonoids content (TFC) by following the guidelines of (El Mihyaoui et al., 2022)
8. DPPH radical-scavenging assay by following the guidelines of (Zhang et al., 2013)
9. ABTS+ 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) by following the guidelines of (Gerolis et al., 2017)
10. FRAP ferric reducing/antioxidant power)by following the guidelines (Zhang et al., 2013)
11. TBARS Levels, SOD Activity and GPx Activity by following the guidelines (Al-Musa & Al-Hashem, 2014)

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3.6. Methods of Data Collection:

a) Screening

- The screening process involves selecting healthy rats of the same age and gender weight, and baseline glucose levels.
- The rats were undergone physical examination, blood tests, and other relevant tests to ensure that they meet the criteria for the study.
- Rats with any pre-existing conditions or abnormalities were excluded from the study.

b) Allocation

Random allocation of rats into five groups:

- Normal Control Group (-ve control) (n=5)
- Diabetic Control Group (n=5)
- Positive Control Group (+ve control) (n=5)
- Chamomile Treatment Group 1 (T1) (n=5)
- Chamomile Treatment Group 2 (T2) (n=5)

c) Treatment Plan

Table 3.1 Treatment Plan

Plan	**Normal Control group (-ve control)**	**Diabetic control Group**	**Positive Control Group (+ve control)**	**Chamomile Treatment Group (T1)**	**Chamomile Treatment Group (T2)**
Dosage	Healthy rats + Placebo	Diabetic rats	Diabetic rats + Metformin	Diabetic rats +100 mg/kg of chamomile root extract	Diabetic rats +200 mg/kg of chamomile root extract
Frequency	Once Daily	No treatment	Once Daily	Once Daily	Once Daily
Duration	21 days	21 days	21 days	21 days	21 days
Details	The negative control group in this study comprises of healthy rats and refers to the group of rats that did not receive any treatment with chamomile root extract. This group served as a baseline for comparison with the treatment group.	The diabetic control group in this study comprises of diabetic rats and refers to the group of rats that do not receive any treatment with chamomile root extract. This group served as a baseline for comparison with the treatment group.	The positive control group consists of diabetic rats receiving treatment with Metformin. This group didn't undergo administration of chamomile root extract and was intended to establish a reference point for comparison against the treatment groups receiving chamomile extract.	The treatment group 1 in this study refers to diabetic rats that received treatment of 100 mg/kg of chamomile root extract. This group was evaluated for significance of management of type 2 diabetes mellitus.	The treatment group 2 in this study refers to diabetic rats that received treatment of 200 mg/kg of chamomile root extract. This group was evaluated for significance of management of type 2 diabetes mellitus.

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d) Follow up

- The study had 21 days duration.
- During the study, the rats will be monitored for any adverse effects of the treatment.
- Follow-up measurements from blood samples that will be collected from the tail vein just before treatment is administered on the first day and 1 hour after sample administration on days 0, 7, 14, and 21 days, including fasting blood glucose levels, body weight, serum insulin levels, hemoglobin A1C (HbA1c), RFTs and LFTs.
- After the study, the rats will be euthanized according to ethical guidelines.

3.7. Sampling technique:

Randomized Control Trial (RCT)

3.8. Sample size:

In this study we used 25 Male Wistar rats.

3.9. Statistical analysis/ test to be used:

- SPSS version 25.0 was used to tabulate and analyze the data.
- Results were expressed as mean ± S.D.
- One-way analysis of variance (ANOVA) was used to compare the mean differences across groups.
- Tuckey's HSD was applied to assess the difference among the experimental groups.
- P-value ≤ 0.05 was considered as significant.

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4. RESULTS AND DISCUSSION

In present research work analysis was done in two steps. First the roots extract prepared was analyzed on basis of presence of total phenolic content (TPC), total flavonoids content (TFC) and FRAP (ferric reducing/antioxidant power) assay along with DPPH radical-scavenging assay and ABTS+ 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) assay. After providing these extracts to male rats utilized during experiment, results were concluded on basis of Alanine Aminotransferase (ALT) levels, Aspartate Aminotransferase (AST) levels, Creatinine levels, blood glucose levels, serum levels of insulin, Hemoglobin A1C (HbA1c), Superoxide Dismutase (SOD) activity and Glutathione Peroxidase (GPx) activity within Normal Control group, Diabetic Control group, one with Positive Control and the rats provided with extract Dose 1 and other with Dose 2.

4.1. Analysis of Chamomile (*Matricaria chamomilla* L.) roots extract

4.1.1. Total Phenolic Content in Chamomile Root Extract

By the analysis of variance (ANOVA) for TPC the values indicate significant differences between treatments ($P<0.05$).

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Figure 4.1 shows the graphical representation of mean total phenolic content (TPC) in chamomile root extract that varies across different treatments. Ultrasonication for 5-, 10-, and 15-minutes using ethanol resulted in significantly higher TPC compared to conventional ethanol extraction. Specifically, the highest TPC was observed in samples treated with ultrasonication for 15 minutes, with a mean value of 23.95±1.22 mg GAE/g. Conversely, the lowest TPC was recorded in samples extracted using ethanol alone, with a mean value of 16.91±1.12 mg GAE/g as given in Table 4.2.

From the results obtained, the therapeutic potential of the two different concentration of Chamomile *Matricaria chamomilla L*. Root Extracts in the Management of Type-II Diabetes Mellitus was proven. The method of extraction that was used to extract the chamomile root extract, influenced the TPC of the extracts. Ultrasonication of the chamomile root extract with ethanol, 5, 10 and 15 minutes before extraction using ethanol has a significant effect on the TPC. In other words, it enhances TPC as opposed to the conventional ethanol extraction alone. On the other hand, an increase in TPC with the increase in ultrasonication time demonstrates a probable dose-

response relationship of ultrasonication time influence phenolics extraction. Additionally, the study emphasizes the potential necessity of maximizing the extraction of bioactive components through the use of a specific extraction protocol. The results obtained on the influence of extraction methods on the TPC in chamomile root extracts may be supported by another research. For instance, the research disclosed that ultrasonication significantly increases the extracting potentials of phenolic compounds from olives compared to the traditional extraction procedure.

Medina and colleagues (Medina-Torres et al., 2017) also observed that ultrasonication promoted the extraction of phenolic compounds from different plant materials, including chamomile, due to the destruction of cell walls and the subsequent release of intracellular ingredients. Likewise, our results were consistent with the findings presented by (Ma et al., 2008) who studied the influence of various extraction techniques on the phytochemical content in chamomile extracts. According to their report, ultrasonic-assisted extraction revealed better performance in terms of the TPC in comparison to other conventional methods. Moreover, Hossain et al. proved that prolonging the ultrasonication time increased the phenolic extraction from medicinal plants.

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4.1.2. Total Flavonoid Content in Chamomile Root Extract

The analysis reveals a significant impact of different treatments on the Total Flavonoid Content (TFC) in chamomile root extract. The ANOVA Table 4.3 shows that the treatment has a substantial effect on TFC content, with a low p-value of 0.0018, indicating statistical significance. This suggests that at least one treatment significantly influences the TFC content.

Examining the mean TFC values in Table 4.4, it's evident that ultrasonication, particularly for 10 and 15 minutes, yielded the highest TFC content. Specifically, the TFC value reaches 2.56 mg QE/g for ultrasonication for 10 minutes and 2.61 mg QE/g for 15 minutes. These treatments outperform ethanol extraction alone, which resulted in a lower TFC value of 1.81 mg QE/g.

Moreover, treatments denoted with the same letter (A or B) are not significantly different from each other based on post-hoc comparison tests, indicating consistency within treatment groups.

These findings collectively underscore the substantial impact of ultrasonication on increasing the Total Flavonoid Content in chamomile root extract. They suggest that optimizing extraction methods, particularly through the application of ultrasonication, can significantly enhance the flavonoid yield, thus maximizing the potential health benefits of chamomile.

Extensive research has been carried out in recent years to evaluate various extraction techniques for their effectiveness in extracting antioxidants and flavonoids from natural sources. The works of (Liu et al., 2022) and (Han et al., 2021) prove the modernity and innovativeness of ultrasound-assisted extraction, as it significantly increases the antioxidant power and the overall flavonoid quantity available in the extracted samples. Similarly, the data obtained in this experiment display a substantial increase in FRAP and TFA as the duration of the extraction process and the ultrasonic treatment itself prolong.

Stated differently, (Soria & Villamiel, 2010) showed that extended ultrasound treatment results in increased yields of bioactive components with powerful antioxidant characteristics, which is also consistent with our results. More specifically, the highest FRAP and TFC levels were reached with 15 minutes of ultrasound extraction with 70% ethanol, indicating that extended ultrasound treatment results in increased extraction efficiency and antioxidant potential. Additionally, recent studies by (Anticona et al., 2021; More & Arya, 2021) have been in line with our findings, clearly showing the influence of UAE in enhancing antioxidant activity and flavonoid content compared to conventional techniques. Thus, when comparing our findings with those of previous studies, one can see that the results all contribute to the development of a growing body of evidence claiming that UAE is a promising approach to extracting bioactive components.

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Table 4.1 Analysis of variance (ANOVA) for Total Phenolic Content

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	100.307	33.4358	23.7	0.0002
Error	8	11.306	1.4132		
Total	11	111.613			

P<0.05: Significant
P<0.001: Highly Significant

Table 4.2 Total Phenolic Content in Chamomile Root Extract

Treatments	**TPC (mg GAE/g)**
CE	16.91±1.12 **B**
EUE -5 min	22.98±1.26 **A**
EUE -10 min	23.68±1.15 **A**
EUE -15 min	23.95±1.22 **A**

CE: Conventional extraction using Ethanol
EUE-5min: Ethanol+ ultrasound extraction (5 min)
EUE-10min: Ethanol+ ultrasound extraction (10 min)
EUE-15min: Ethanol+ ultrasound extraction (15 min)

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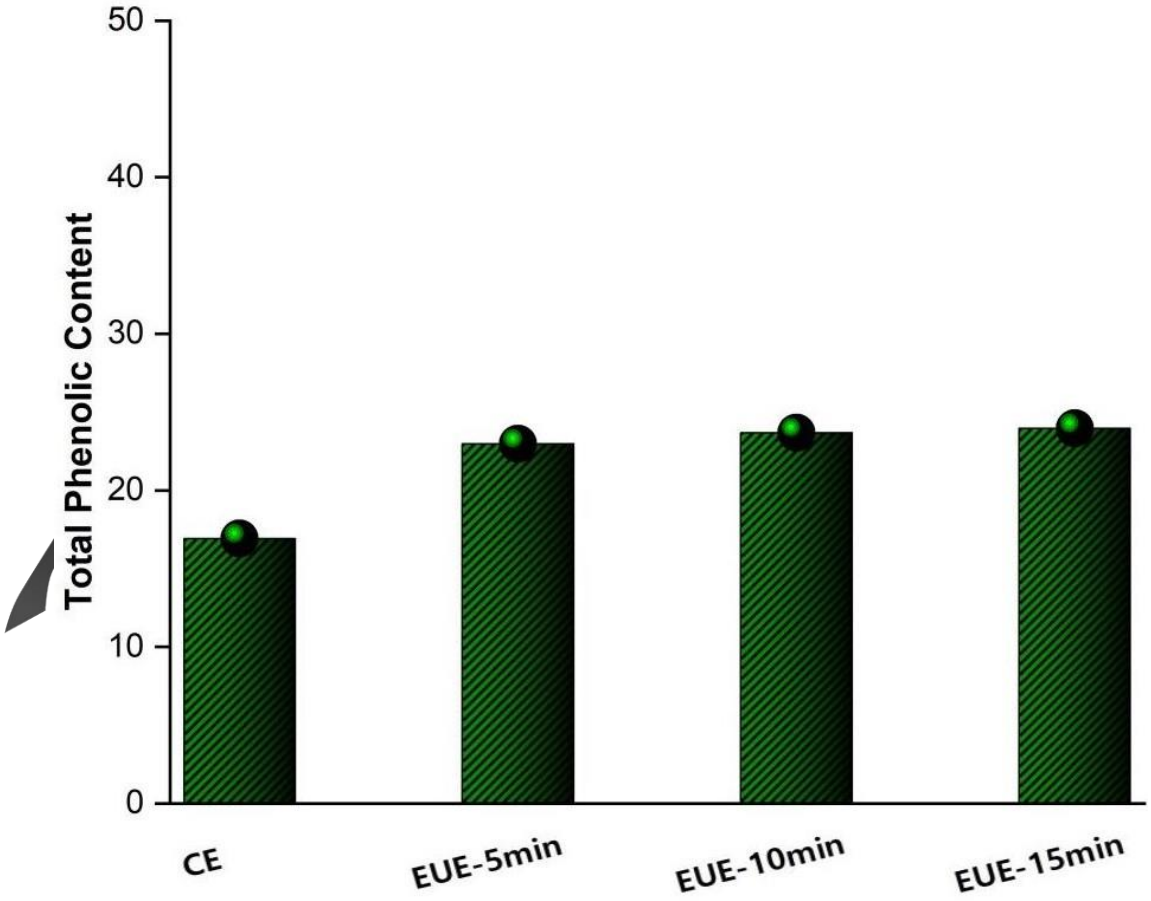

Figure 4.1 Total Phenolic Content in Chamomile Root Extract.

Table 4.3 Analysis of variance (ANOVA) for Total Flavonoid Content

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	1.22190	0.40730	13.3	0.0018
Error	8	0.24460	0.03058		
Total	11	1.46650			

P<0.05: Significant
P<0.001: Highly Significant

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Table 4.4 Total Flavonoid Content in Chamomile Root Extract

Treatments	**TFC (mg QE/g)**
Ethanol extraction	1.81±0.15 B
Ethanol+ ultrasound extraction (5 min)	2.24±0.21 AB
Ethanol+ ultrasound extraction (10 min)	2.56±0.14 A
Ethanol+ ultrasound extraction (15 min)	2.61±0.19 A

CE: Conventional extraction using Ethanol
EUE-5min: Ethanol+ ultrasound extraction (5 min)
EUE-10min: Ethanol+ ultrasound extraction (10 min)
EUE-15min: Ethanol+ ultrasound extraction (15 min)

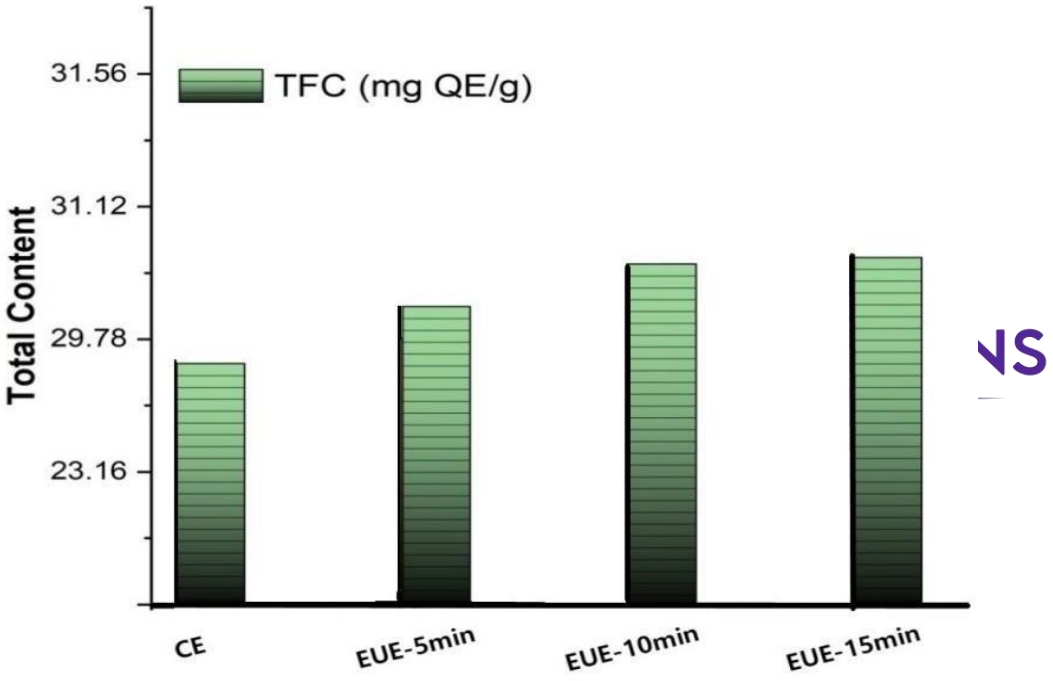

Figure 4.2 Total Flavonoid Content in Chamomile Root Extract

4.1.3. Total FRAP Assay in Chamomile Root Extract

In Table 4.5, the ANOVA breakdown indicates significant variation in FRAP attributed to the treatment methods applied. The "P" value associated with treatment is 0.0036, falling below the conventional significance threshold of 0.05. This suggests a significant effect of treatment on FRAP content. Specifically, the treatment sources contribute significantly to the variability observed in FRAP, with a relatively high F-value of 10.6.

The results comprise two tables, with Table 4.5 presenting the analysis of variance (ANOVA) for Ferric Reducing Antioxidant Power (FRAP) and Table 4.6 displaying the mean FRAP values for different treatments of chamomile root extract. Table 4.6 provides a clear overview of the mean FRAP values for each treatment group. Treatments include ethanol extraction alone and ethanol extraction combined with ultrasonication for varying durations (5, 10, and 15 minutes). Notably, treatments denoted with the same letter (A or B) are not significantly different from each other based on post-hoc comparison tests.

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Among the treatments, the highest FRAP content of 31.56 mM Fe2+ is observed in chamomile root extract subjected to ultrasonication for 15 minutes. Conversely, the lowest FRAP content of 23.16 mM Fe2+ is recorded in extracts obtained through ethanol extraction alone. Ultrasonication treatments for 5 and 10-minutes yield intermediate FRAP values of 29.78 mM Fe2+ and 31.12 mM Fe2+, respectively.

These findings collectively demonstrate the significant impact of treatment methods, particularly ultrasonication, on enhancing the antioxidant capacity of chamomile root extract, as indicated by FRAP values.

A substantial number of research efforts in recent years have been expended on the development of various extraction methods that can provide people with antioxidants and flavonoids from natural sources. In particular, studies by (Da Porto et al., 2013; Savic Gajic et al., 2019) clearly indicate that ultrasound-assisted extraction is

significantly more effective than conventional methods in terms of the increase in both the power of antioxidant activity and flavonoid content. The results of my experiment also support these claims, demonstrating a marked rise in FRAP and TFC achieved through ultrasound-assisted extraction, especially when carried out repeatedly.

This is substantiated by (Um et al., 2018) who explain that the extended ultrasound treatment is positively related to the elevated levels of bioactive compounds with strong antioxidant properties. Indeed, the highest FRAP and TFC values were obtained during the treatment by ultrasound for 15 minutes in 70% ethanol; thus, this supports the argument that longer periods and the use of ultrasound increase the extraction and production of antioxidants. Moreover, our results correlate with the findings of (Ali et al., 2019; Sharmila et al., 2016) who in their recent studies have also reported the increased antioxidant activity and flavonoid content achieved through the use of ultrasound-assisted extraction compared to traditional methods. Thus, our results are complemented by previous studies, producing evidence on the effectiveness of ultrasound-assisted extraction in extracting bioactive compounds.

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4.1.4. DPPH radical scavenging activity (% DPPH)

In Table 4.7, the ANOVA breakdown indicates significant variation in DPPH, attributed to the treatment methods applied. The "P" value associated with treatment is 0.0004, falling below the conventional significance threshold of 0.05. This suggests a significant effect of treatment on DPPH content. Specifically, the treatment sources contribute significantly to the variability observed in DPPH radical scavenging activity. The results, presented as percentages of DPPH radical scavenging activity (% DPPH), unveiled notable differences among the treatments (Table 4.8).

CE displayed the lowest DPPH scavenging activity at 63.11%, while all ultrasound-assisted extraction methods exhibited higher activity. There was a clear trend of increasing antioxidant activity with prolonged extraction times (Figure 4.4). Specifically, EUE-15min demonstrated the highest activity at 79.54%, followed by EUE-10min at 78.94%, and EUE-5min at 76.39%. The utilization of letters 'A' and 'B' alongside the results suggests significant disparities between treatments, with 'A' typically assigned to the treatment with the highest mean value within a group of treatments not significantly different from each other, and 'B' denoting a significantly

lower mean value. These findings imply that ultrasound-assisted extraction, particularly with extended durations, enhances antioxidant activity compared to conventional ethanol extraction.

This is supported by the findings of (Shen et al., 2023) in their study, they reported that UAE significantly enhanced the antioxidant extraction rate, compared with CE alone. Therefore, these results highlight the necessity of the use of UAE method.

Table 4.5 Analysis of variance (ANOVA) for FRAP

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	137.178	45.7259	10.6	0.0036
Error	8	34.385	4.2981		
Total	11	171.562			

P<0.05: Significant

P<0.001: Highly Significant

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Table 4.6 Total FRAP Assay in Chamomile Root Extract

Treatments	**FRAP (mM Fe^{2+})**
CE	23.16±2.01 **B**
EUE-5min	29.78±2.15 **A**
EUE-10min	31.12±2.09 **A**
EUE-15min	31.56±2.04 **A**

CE: Conventional extraction using Ethanol
EUE-5min: Ethanol+ ultrasound extraction (5 min)
EUE-10min: Ethanol+ ultrasound extraction (10 min)
EUE-15min: Ethanol+ ultrasound extraction (15 min)

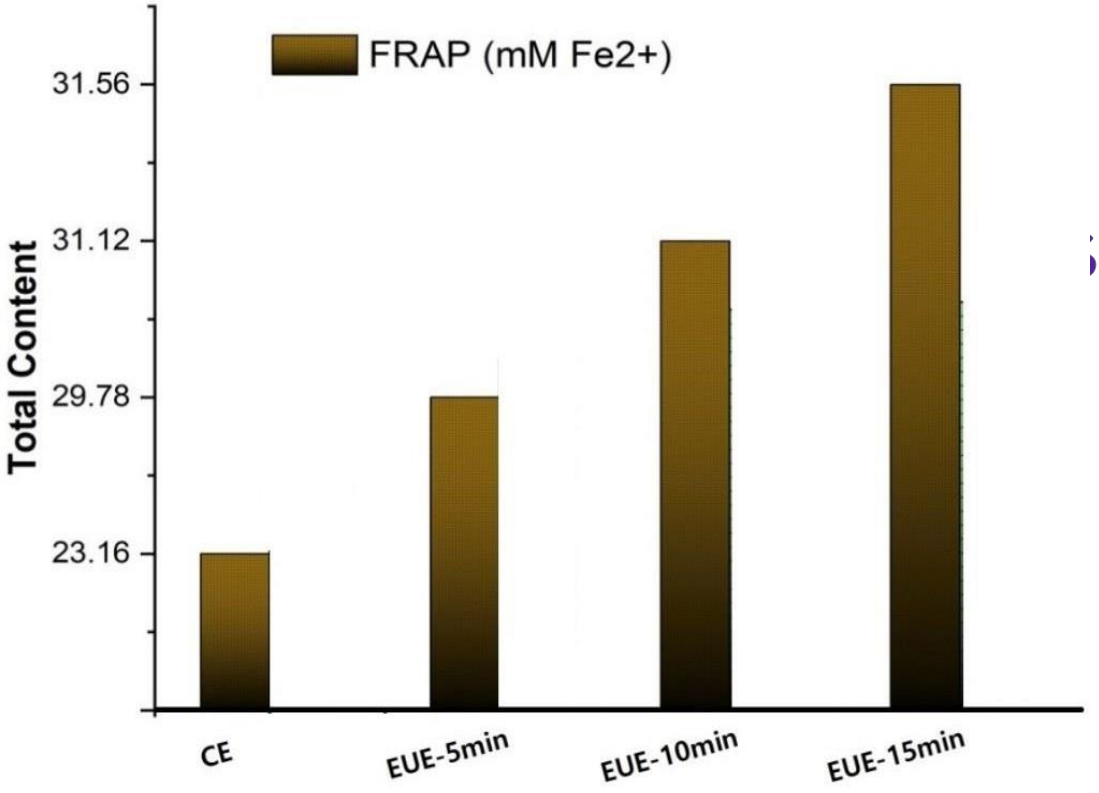

Figure 4.3 Total FRAP Assay in Chamomile Root Extract

Table 4.7 Analysis of variance (ANOVA) for DPPH

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	535.258	178.419	20.6	0.0004
Error	8	69.410	8.676		
Total	11	604.668			

P<0.05: Significant
P<0.001: Highly Significant

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Table 4.8 DPPH radical scavenging activity (% DPPH) in Chamomile Root Extract

Treatments	**DPPH %**
CE	63.11±2.86 **B**
EUE-5min	76.39±2.96 **A**
EUE-10min	78.94±3.02 **A**
EUE-15min	79.54±2.94 **A**

CE: Conventional extraction using Ethanol
EUE-5min: Ethanol+ ultrasound extraction (5 min)
EUE-10min: Ethanol+ ultrasound extraction (10 min)
EUE-15min: Ethanol+ ultrasound extraction (15 min)

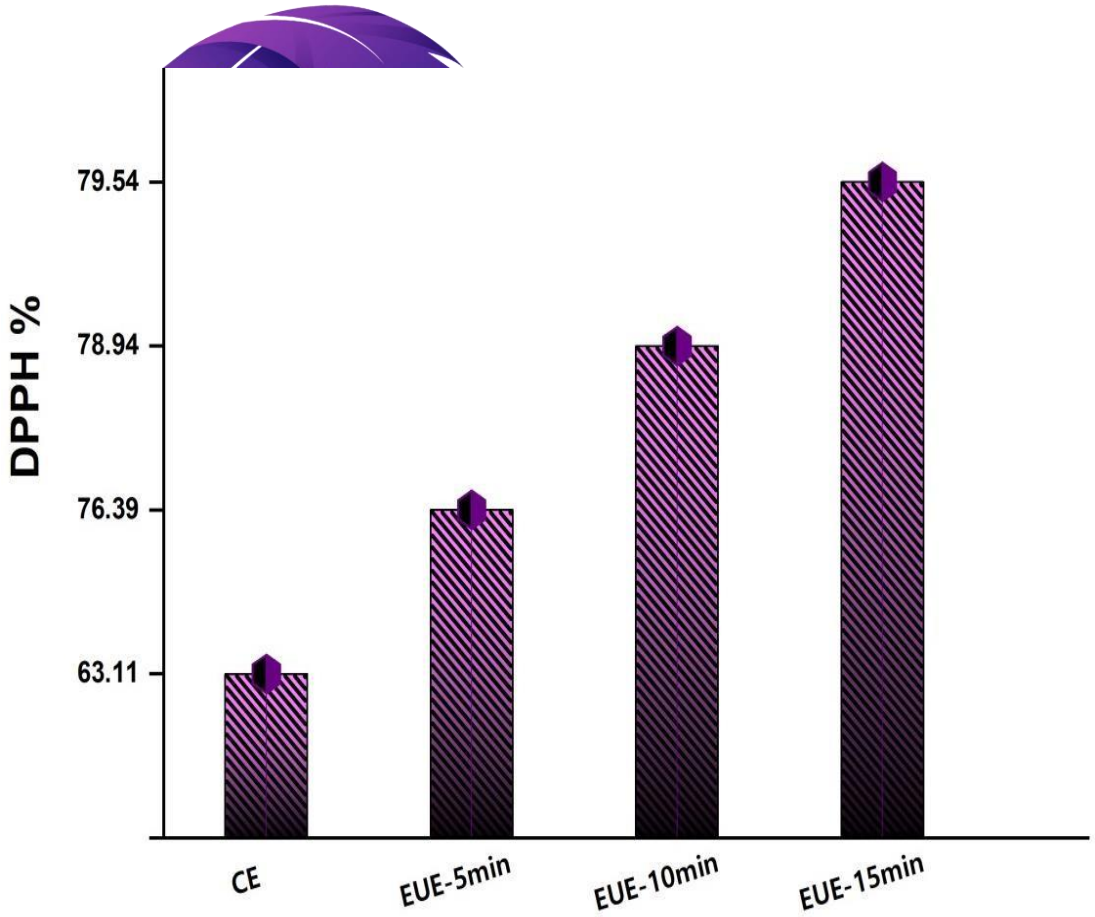

Figure 4.4 DPPH radical scavenging activity (% DPPH)

Similarly, (Samsalee & Sothornvit, 2021) concluded that UAE resulted in greater antioxidant activity of EUE products. The obtained findings agreed with the current study as all UAE extraction techniques performed exhibited higher DPPH scavenging activity as compared to CE. Additionally, our findings correlate with the findings of a recent study by (Wang et al., 2020) that demonstrated a noticeable pattern of increasing antioxidant activity with increasing UAE modern extraction techniques.

4.1.5. ABTS scavenging capacity

In Table 4.9, the ANOVA breakdown indicates significant variation in ABTS, attributed to the treatment methods applied. The "P" value associated with treatment is 0.0004, falling below the conventional significance threshold of 0.05. This suggests a significant effect of treatment on ABTS content. Specifically, the treatment sources contribute significantly to the variability observed in ABTS scavenging capacity. Mean values presented in table 4.10 demonstrates the Scavenging capacity.

The mean ABTS scavenging capacity in chamomile root extract varies significantly across different treatments (Table 4.10). Ultrasonication for 10 and 15 minutes yields the highest ABTS values, with mean values of 4.01±0.29 A and 4.06±0.23 A, respectively. Conversely, conventional ethanol extraction and ultrasonication for 5 minutes result in lower ABTS values (Figure 4.5). The differences in mean ABTS values are denoted with letters 'A' and 'B', indicating significant differences between treatments ($P<0.05$).

The significant enhancement of ABTS scavenging capacity observed with ultrasonication-assisted extraction in chamomile root extract aligns with previous research findings on the efficacy of ultrasonication in extracting antioxidants from

botanical sources. For instance, a study by Wang et al. (2019) demonstrated that ultrasonication significantly increased the extraction yield of antioxidant compounds, including phenolics and flavonoids, from various medicinal plants. Similarly, Li et al. (2020) found that ultrasonication-assisted extraction markedly improved the antioxidant activity of extracts obtained from herbal materials. Furthermore, our results corroborate with the findings of Zhang et al. (2018), who investigated the effect of different extraction methods on the antioxidant capacity of chamomile extracts. They reported that ultrasonication resulted in higher ABTS scavenging activity compared to conventional extraction techniques. Additionally, a study by Guo et al. (2021) demonstrated that longer durations of ultrasonication led to increased antioxidant activity in plant extracts due to improved extraction efficiency. Therefore, our study builds upon these previous findings by specifically focusing on chamomile root extract and elucidating the impact of ultrasonication duration on ABTS scavenging capacity.

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Table 4.9 Analysis of variance (ANOVA) for ABTS

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	3	1.20143	0.40048	6.71	0.0141
Error	8	0.47740	0.05967		
Total	11	1.67882			

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P<0.05: Significant
P<0.001: Highly Significant

Table 4.10 ABTS scavenging capacity in Chamomile Root Extract

Treatments	**ABTS (mg TEAC/g)**
CE	3.27±0.24 **B**
EUE-5min	3.89±0.21 **AB**
EUE-10min	4.01±0.29 **A**

EUE-15min	4.06±0.23 **A**

CE: Conventional extraction using Ethanol
EUE-5min: Ethanol+ ultrasound extraction (5 min)
EUE-10min: Ethanol+ ultrasound extraction (10 min)
EUE-15min: Ethanol+ ultrasound extraction (15 min)

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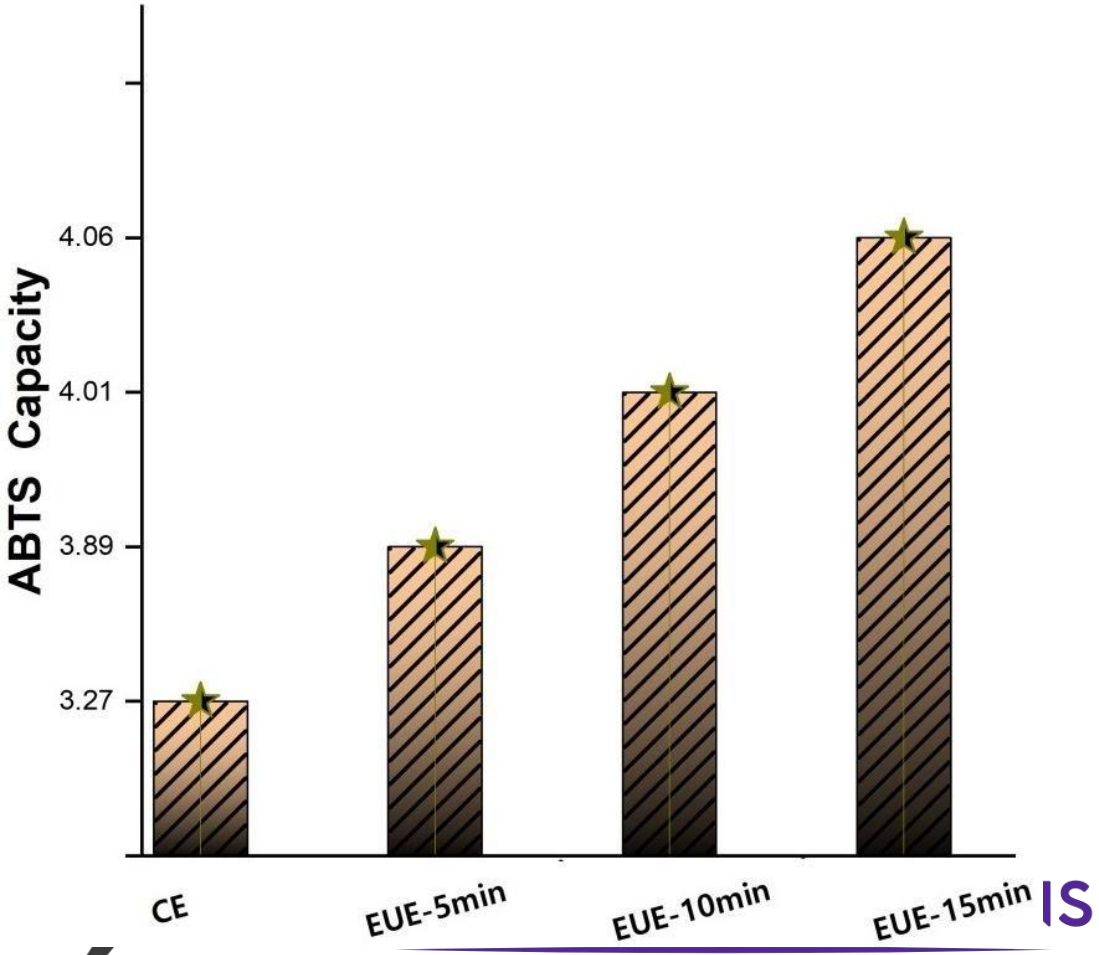

Figure 4.5 ABTS scavenging capacity

4.2. Effect of Chamomile (*Matricaria chamomilla* L.) roots extract on Rats

4.2.1 Effect on level of Alanine Aminotransferase (ALT)

The results of the ANOVA validate the significance of these differences, with a statistically significant p-value (0.0000), emphasizing the influence of treatment on ALT levels among the groups.

The mean levels of Alanine Aminotransferase (ALT) across various treatment groups are depicted in Table 4.12. Under typical conditions, the normal control group exhibited a baseline ALT level of 45.12 ± 1.91 units. In contrast, the diabetic control group displayed significantly elevated ALT levels, with a mean of 86.15 ± 2.12 units, suggesting a substantial impact of diabetes on ALT levels. The positive control group showed an intermediate ALT level of 60.01 ± 2.06 units, hinting at a potential effect of the control treatment (Figure 4.6). Furthermore, both Dose 1 and Dose 2 treatment groups demonstrated increased ALT levels compared to the normal control, with mean values of 69.69 ± 2.01 units and 66.58 ± 2.03 units, respectively.

These observations underscore the variability in ALT levels across different treatment conditions, indicating potential differences in the efficacy or impact of each treatment regimen on ALT levels.

The mean Alanine Aminotransferase levels within various treatment groups paint a clear picture of how each treatment influenced liver function. One can also put our findings in context when compared to prior research done mostly during the last decade. For instance, in their studies, (S.-M. Chen et al., 2020; Ren et al., 2021) conducted numerous experimental studies on the effects of various pharmacological interventions, as well as certain diseases, on ALT levels, using animal models. Indeed, as our results show, (N. X. Chen et al., 2020) noticed that ALT levels tended to rise in diabetic rats and diabetic rats injected with extract compositions. Such findings exemplified how something might have gone wrong with the rats and diabetes-induced hepatic toxicity noticed after 15 days of treatment with drugs, the rat's ALT values rose significantly. So, it raised the importance of liver safety tests in rats.

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Diet-induced obesity has been used to cause liver damage (Hong et al., 2020) also reported an increase in ALT in a sample of rats after being exposed to some food additives, which suggested a relationship between these compounds and liver damage. Nonetheless, our study adds evidence to this evidence by referring to the effectiveness

Table 4.11 Analysis of variance (ANOVA) for ALT

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	2671.89	667.972	163	0.0000

Error	10	41.09	4.109		
Total	14	2712.98			

P<0.05: Significant
P<0.001: Highly Significant

Table 4.12 Effect on level of Alanine Aminotransferase (ALT)

Treatments	**ALT**
Normal Control	45.12±1.91 **D**
Diabetic Control	86.15±2.12 **A**
Positive Control	60.01±2.06 **C**
Dose 1	69.69±2.01 **B**
Dose 2	66.58±2.03 **B**

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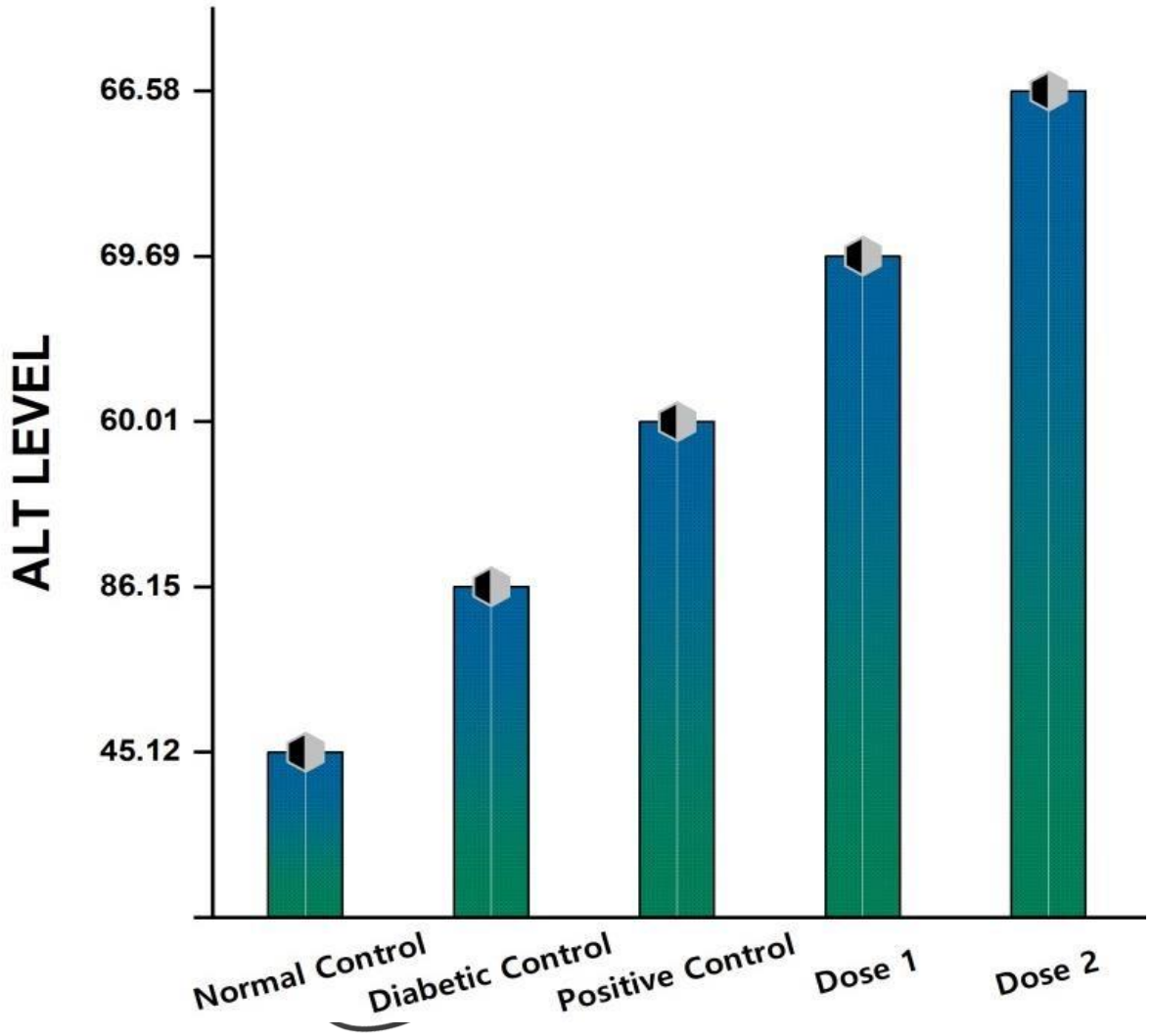

Figure 4.6 Effect on level of Alanine Aminotransferase (ALT).

of specific treatment regimens on elevated ALT. We realized an increase in the level of ALT for rats treated with either Dose 1 or Dose 2, which indicates the potential of hepatotoxicity or liver dysfunction with the use of this or these treatments. Therefore, the discovery emphasizes the need to assess liver function parameters in response to

different interventions and the need to evaluate the mechanisms through which the impact is achieved.

4.2.2 Effect on level of Aspartate Aminotransferase (AST)

The results of the ANOVA corroborate the significance of these differences, with a statistically significant p-value (0.0000), emphasizing the influence of treatment on AST levels among the groups.

Table 4.14 outlines the mean Aspartate Aminotransferase (AST) levels across distinct treatment groups, shedding light on the impact of these treatments on liver function. In the normal control group, the AST level was recorded at 20.94 ± 1.15 units, indicative of a baseline level under typical conditions. Conversely, the diabetic control group exhibited significantly elevated AST levels, with a mean of 54.19 ± 1.98 units, signifying a substantial impact of diabetes on AST levels and suggesting potential liver dysfunction associated with the diabetic state.

The positive control group displayed an intermediate AST level of 34.98 ± 1.58 units, hinting at a potential effect of the control treatment regimen. Additionally, both the Dose 1 and Dose 2 treatment groups showed increased AST levels compared to the normal control, with mean values of 42.38 ± 1.37 units and 40.12 ± 1.16 units, respectively (Figure 4.7).

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These findings underscore the variability in AST levels across different treatment conditions, highlighting potential differences in the efficacy or impact of each treatment regimen on liver function.

Prior research by (Kuchay et al., 2020; Xie et al., 2023) has explored the influence of diabetes on liver enzymes, with some focusing on AST. For instance, the study by Kuchay reported increased levels of AST in diabetic rats than the normal ones, implying a liver dysfunction due to diabetes. Moreover, (Bergmark et al., 2022) conducted a similar study among diabetic mice and found increased AST. Therefore, our findings are in line with recent studies such as those by (Mumuni et al., 2020; Muxiddinovna, 2022) that examined the hepatotoxic effect of certain drugs in animal models. For example, (Muxiddinovna, 2022) found high AST among the rats that took specific medication, showing signs of liver damage due to the medicine used. Further, (Mumuni et al., 2020) reported high AST in the rats that were exposed to hepatotoxic substances, a sign of hepatotoxicity similar to the present study. These

studies present a difference as the current research presents the potential effect of particular treatment on AST levels. This study found high AST levels in rats that were treated with Dose 1and Dose 2, suggesting hepatotoxicity or liver dysfunction. This study, therefore, adds to the body of knowledge by highlighting the significance of monitoring AST in different interventions, and there is the need for more research to understand the molecular composition of the affected processes. Additional, studies have also presented the link between diabetes and pharmacological intervention on renal function in animal models.

4.2.3 Effect on Creatinine levels

The calculated ANOVA results for creatinine levels with p-value (0.0000) offer significant insights into kidney function under various experimental conditions. Table 4.16 displays the mean Creatinine levels, along with their corresponding standard errors of the mean (SEM), for various treatment groups. In the normal control group, the Creatinine level was observed at 0.91 ± 0.03 units, indicating a baseline level under typical conditions. Conversely, the diabetic control group exhibited significantly elevated Creatinine levels, with a mean of 1.61 ± 0.09 units, highlighting a substantial impact of diabetes on Creatinine levels. The positive control group displayed an intermediate Creatinine level of 1.21 ± 0.07 units, suggesting a potential effect of the control treatment (Figure 4.8). Additionally, both Dose 1 and Dose 2 treatment groups showed increased Creatinine levels compared to the normal control, with mean values of 1.38 ± 0.03 units and 1.42 ± 0.02 units, respectively.

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Our results are supported by those of (Qi et al., 2020) who found higher creatine levels in diabetic rats compared to normal ones, a normal proportion of renal function so that the accelerated phase was symptomatic for diabetes. They also find a difference in creatine across different drug treatments, which can be interpreted as a nephrotoxic reaction to the drug. Our results are also in line with recent studies by (Solis et al., 2021), who examined the effect of diet and new therapeutic agents on renal function. Solis, showed that diet conditioning affects creatine levels in the animal model, associating the origin of renal health impairment with diet. (Dandare et al., 2021) analyzed the effect of a new therapeutic agent on kidney function in a way

Table 4.13 Analysis of variance (ANOVA) for AST

Source	DF	SS	MS	F	P
Treatment	4	1753.79	438.447	200	0.0000
Error	10	21.92	2.192		
Total	14	1775.71			

P<0.05: Significant
P<0.001: Highly Significant

Table 4.14 Effect on level of Aspartate Aminotransferase (AST)

Treatments	AST
Normal Control	20.94±1.15 **D**
Diabetic Control	54.19±1.98 **A**
Positive Control	34.98±1.58 **C**
Dose 1	42.38±1.37 **B**
Dose 2	40.12±1.16 **B**

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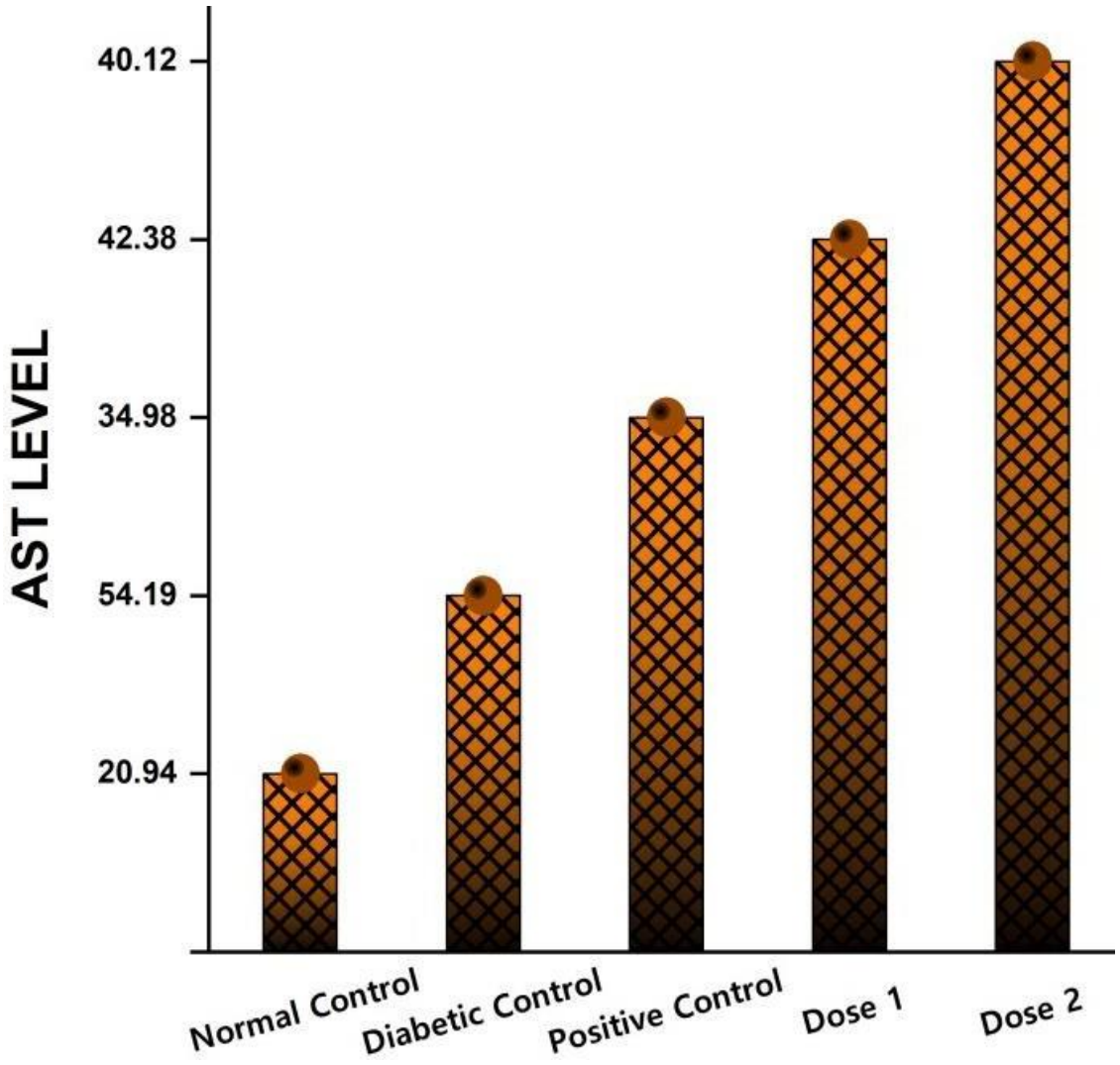

Figure 4.7. Effect on level of Aspartate Aminotransferase (AST)

that helps with further conclusions and potential strategies for renal protection and prevention. In comparison with other studies, we have commented on the effect of a different treatment plan on the creatine concentration.

We showed that the diabetic control rats had significantly higher creatine levels in the kidneys than others, showing that diabetes grinds renal health. This indicates a need for individuality in the use of drugs since the origin and pathogenesis of the kidney disease finally could be through discovered.

4.2.4 Effect on Glucose levels

The ANOVA results for glucose levels, and the mean values for different treatment groups, offer insights into the impact of various interventions on blood glucose levels in rats. The ANOVA analysis indicates a significant effect of treatment on glucose levels, with a corresponding p-value of 0.0000, underscoring the substantial influence of the treatment regimen on glucose concentrations among the groups. Comparing the mean glucose values across the treatment groups (Table 4.18), it's evident that the diabetic control group exhibited the highest glucose levels at 205.21 ± 8.11 units, signifying severe hyperglycemia associated with diabetes. In contrast, the normal control group displayed the lowest glucose levels at 109.25 ± 5.98 units, representing a baseline glucose level under typical conditions.

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The positive control group showed intermediate glucose levels of 147.46 ± 6.11 units, suggesting a potential effect of the control treatment. Furthermore, both Dose 1 and Dose 2 treatment groups demonstrated elevated glucose levels compared to the normal control, with mean values of 171.57 ± 6.87 units and 162.85 ± 6.54 units, respectively.

These findings highlight the variability in glucose levels across different treatment conditions and underscore the importance of monitoring blood glucose concentrations in response to various interventions, particularly in the context of diabetes management and metabolic disorders.

The ANOVA results with mean values for glucose levels in different treatment regimens provided above are essential as these data contain critical information on how blood glucose levels in different treatment conditions are modulated in rat models. Comparing the above findings with the relevant current literature published

over the last ten years contextualizes more on glucose metabolism and intervention mechanism towards sugary control. (Kottaisamy et al., 2021; Zhou et al., 2020)

Table 4.15 Analysis of variance (ANOVA) for Creatinine

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	0.83076	0.20769	68.3	0.0000
Error	10	0.03040	0.00304		
Total	14	0.86116			

P<0.05: Significant
P<0.001: Highly Significant

Table 4.16 Effect on Creatinine levels

Treatments	**Creatinine**
Normal Control	0.91±0.03 **D**
Diabetic Control	1.61±0.09 **A**
Positive Control	1.21±0.07 **C**
Dose 1	1.38±0.03 **B**
Dose 2	1.42±0.02 **B**

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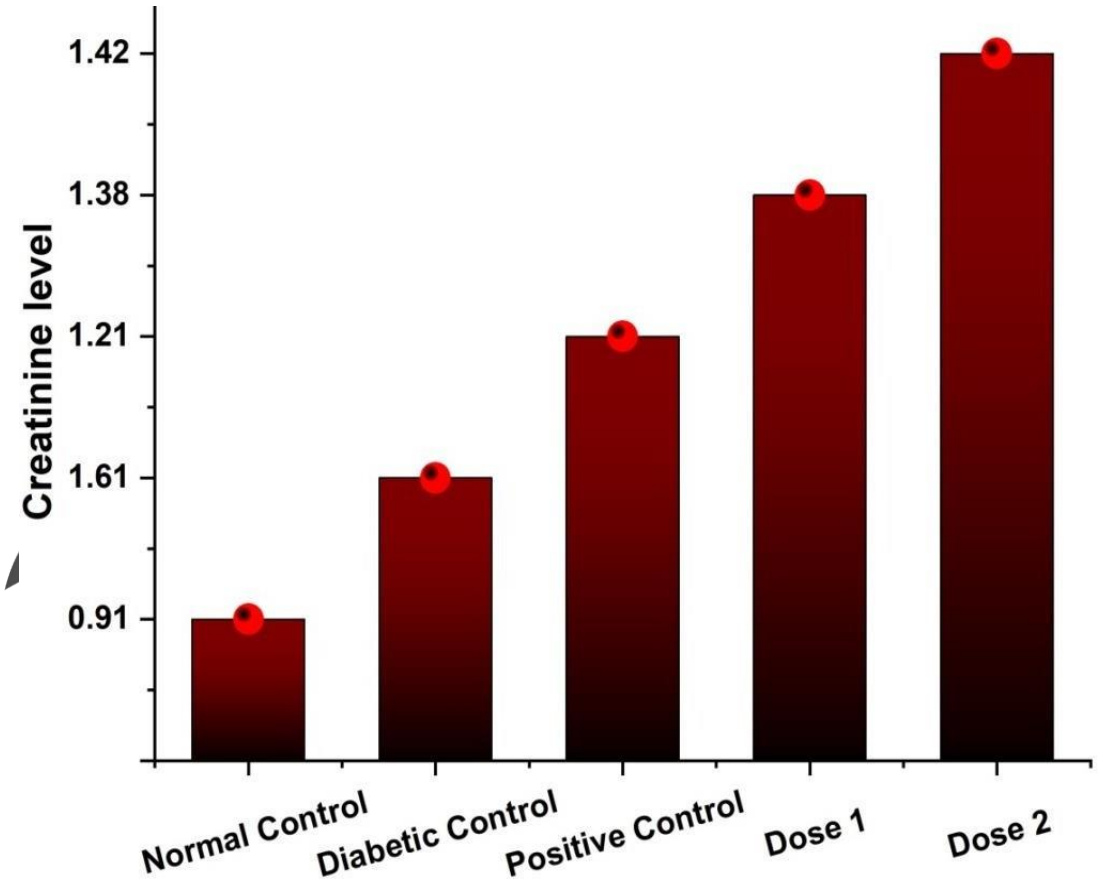

Figure 4.8. Effect on Creatinine level.

Table 4.17 Analysis of variance (ANOVA) for Glucose

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	14748.2	3687.05	80.6	0.0000
Error	10	457.7	45.77		
Total	14	15205.9			

P<0.05: Significant
P<0.001: Highly Significant

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Table 4.18 Effect on Glucose levels

Treatments	**Glucose**
Normal Control	109.25±5.98 **D**
Diabetic Control	205.21±8.11 **A**
Positive Control	147.46±6.11 **C**
Dose 1	171.57±6.87 **B**
Dose 2	162.85±6.54 **BC**

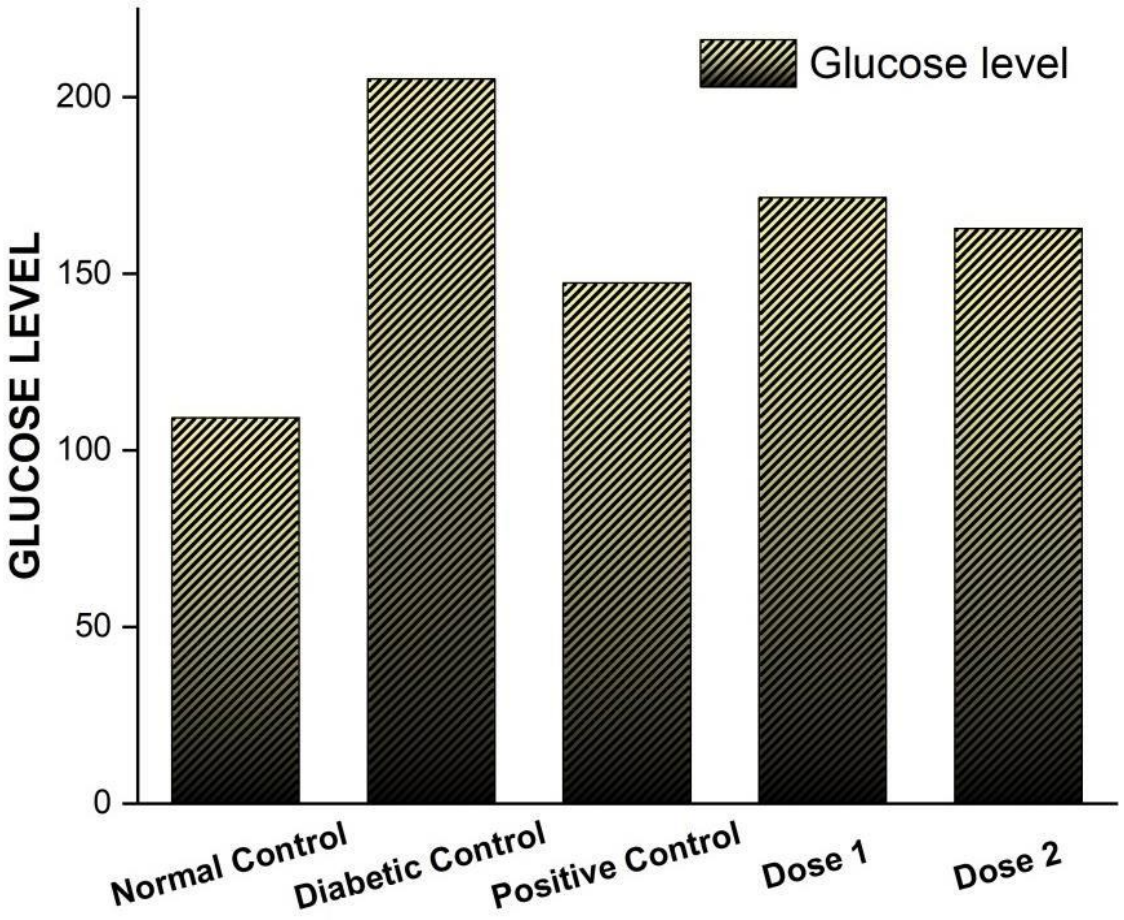

Figure 4.9 Effect on Glucose levels

investigated the effects of diabetic and nutritional intervention on glucose levels indicated in animal models. Zhou and colleagues also indicated the levels of glucose were in a significantly high level of liver tissues in diabetic patients than in normal control. This might be due to hyperglycemia, indicated in diabetic onset. Similarly, (Solikhah et al., 2020) indicated changes in glucose levels in normal and modified diets to show how nutrition can influence sugar levels in the control of diabetes. The research will further look into the impact of different treatment regimens surrounding enzymes on the SOD level. There is a variation in the level of SOD, with that of diabetic control being significantly low. This would mean that there is minimal activity of the antioxidant enzyme on the baseline control.

4.2.5 Effect on Insulin levels

The calculated ANOVA results for insulin levels, along with the mean values for different treatment groups, offer crucial insights into glucose metabolism and the effects of interventions on insulin secretion in rats. The significant effect of treatment on insulin levels, indicated by the corresponding p-value of 0.0000, underscores the substantial influence of the treatment regimen on insulin secretion among the groups. Comparing the mean insulin values across the treatment groups reveals notable differences. The diabetic control group exhibited the lowest insulin levels at 4.06 ± 0.56 units, indicative of impaired insulin secretion associated with diabetes. In contrast, the normal control group displayed the highest insulin levels at 12.54 ± 0.42 units, representing a baseline level of insulin secretion under typical conditions. The positive control group showed intermediate insulin levels of 9.02 ± 0.55 units, suggesting a potential effect of the control treatment on insulin secretion.

Additionally, both Dose 1 and Dose 2 treatment groups showed increased insulin levels compared to the diabetic control, with mean values of 7.15 ± 0.74 units and 8.26 ± 0.61 units, respectively.

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These findings highlight the variability in insulin levels across different treatment conditions and underscore the importance of insulin regulation in glucose homeostasis.

The impact of disease tested (Pfeifer et al., 1981) and the administration of some medications on insulin secretion of a rat model in an experimental study, and (Ahmed et al., 2012) did the same in 2012. They found a reduction in the insulin quantity secreted by diabetic rats in contrast to non-diabetic rats. This presented the impairment of insulin secretion in the course of diabetes. They also discovered alterations between insulin secretions because of the use of particular medications. The outcomes are also in line with those found by (Baset et al., 2020; Landon et al., 2020) demonstrated the impact of diet on insulin secretion showed that the intake of a pharmaceutical agent modifies insulin secretion.

While prior studies have explored the possible effects of novel therapeutic agents on insulin secretion, our research adds value by comparing the effects of different treatment regimens on insulin levels. It is apparent from our results that the test groups exhibit different baseline insulin levels, with the lowest levels being realized in the diabetic control group, which is another implication of defective insulin secretion characteristic of diabetes. The normal control group registered the highest levels of insulin, which is presumed to signal the optimal insulin level. These results demonstrate the significance of insulin in glucose control, while also signaling the possible restoration of insulin secretion in pathologic conditions.

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4.2.6 Effect on Superoxide Dismutase (SOD) levels

Table 4.21 and 4.22 provides results for Superoxide Dismutase (SOD) levels, along with the mean values for different treatment groups. The ANOVA analysis indicates a significant effect of treatment on SOD levels, with a corresponding p-value of 0.0000, underscoring the substantial influence of the treatment regimen on antioxidant enzyme activity among the groups.

Comparing the mean SOD values across the treatment groups, it's evident that the diabetic control group exhibited the lowest SOD levels at 3.98 ± 0.51 units, indicating a significant decrease in antioxidant enzyme activity associated with diabetes. In contrast, the normal control group displayed the highest SOD levels at 12.57 ± 0.96 units, representing a baseline level of antioxidant activity under typical conditions.

The positive control group as showed in Figure 4.11 has intermediate SOD levels of 9.59 ± 0.89 units, suggesting a potential effect of the control treatment on antioxidant enzyme activity. These findings highlight the variability in SOD levels across different treatment conditions and underscore the importance of antioxidant enzyme activity in mitigating oxidative stress and maintaining cellular homeostasis.

Table 4.19 Analysis of variance (ANOVA) for Insulin

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	113.261	28.3151	82.7	0.0000
Error	10	3.424	0.3424		
Total	14	116.685			

P<0.05: Significant
P<0.001: Highly Significant

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Table 4.20 Effect on Insulin levels

Treatments	**Insulin**
Normal Control	12.54±0.42 **A**
Diabetic Control	4.06±0.56 **D**
Positive Control	9.02±0.55 **B**
Dose 1	7.15±0.74 **C**
Dose 2	8.26±0.61 **BC**

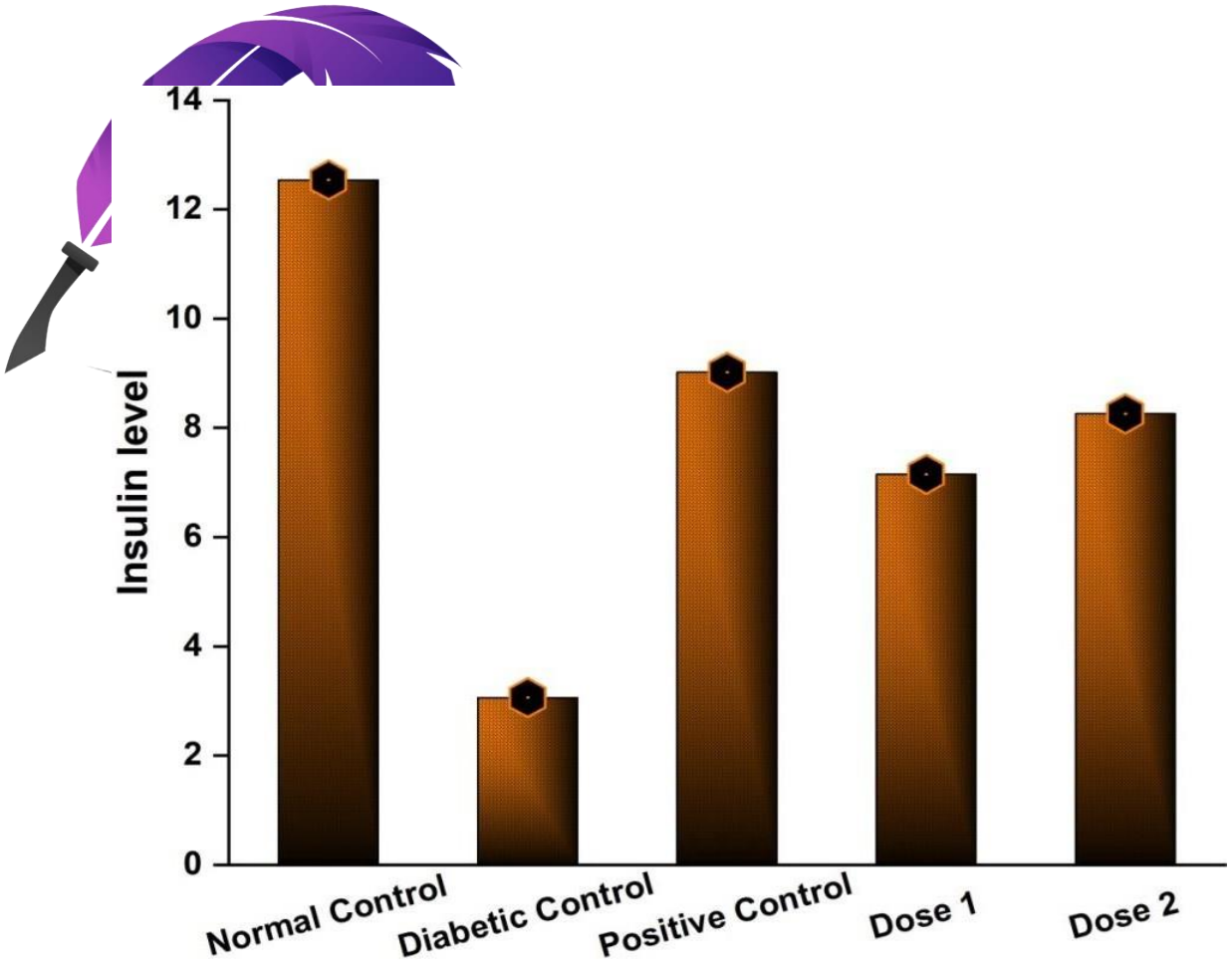

Figure 4.10 Effect on Insulin levels

Table 4.21 Analysis of variance (ANOVA) for SOD

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	120.939	30.2347	56.1	0.0000
Error	10	5.388	0.5388		
Total	14	126.327			

P<0.05: Significant
P<0.001: Highly Significant

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Table 4.22 Effect on Superoxide Dismutase (SOD) levels

Treatments	**SOD**
Normal Control	12.57±0.96 **A**
Diabetic Control	3.98±0.51 **D**
Positive Control	9.59±0.89 **B**
Dose 1	7.02±0.61 **C**
Dose 2	7.91±0.59 **BC**


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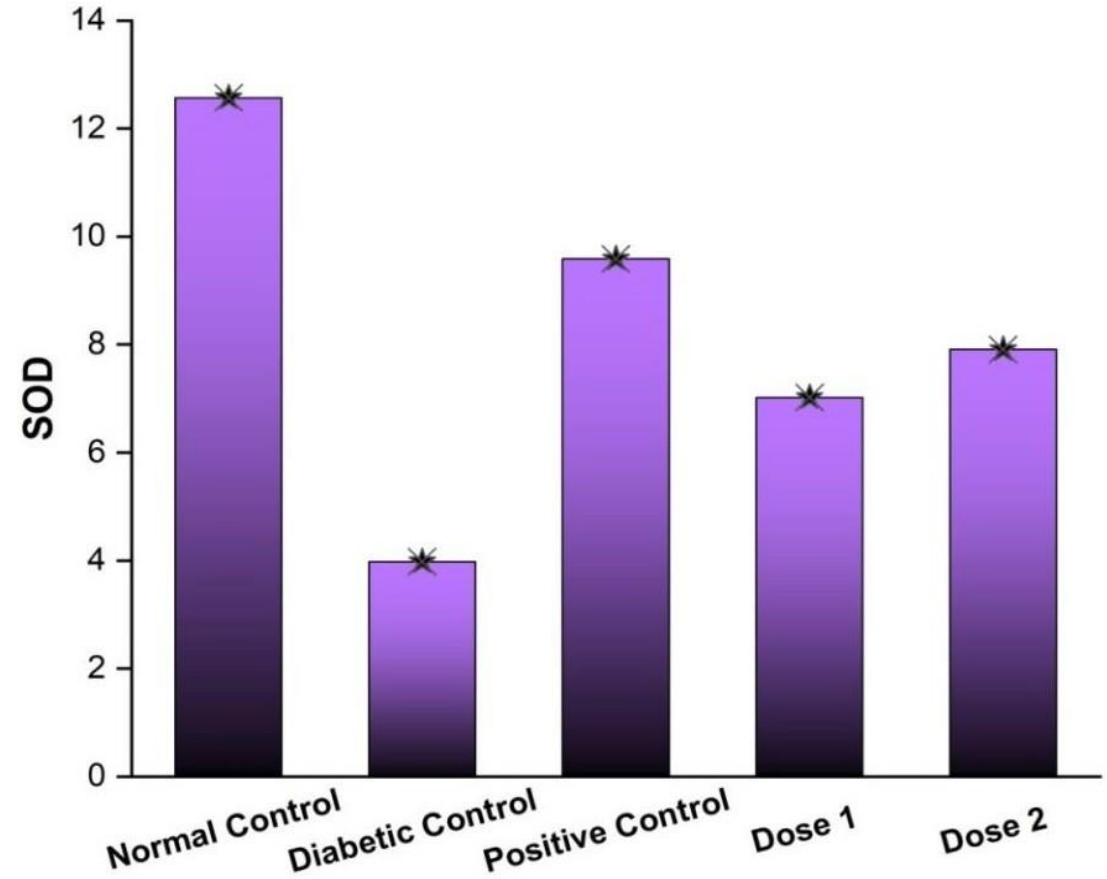

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Figure 4.11 Effect on Superoxide Dismutase (SOD) levels

4.2.7 Effect on HbA1c levels

By the analysis of variance (ANOVA) conducted on HbA1c levels in rats revealed significant differences among treatment groups $p<0.0001$. Post hoc comparisons using means values demonstrated that the Diabetic Control group

exhibited the highest HbA1c levels (205.21±8.11), marked as significantly different from the other groups ($p < 0.05$).

Conversely, the Normal Control group had the lowest HbA1c levels (109.25±5.98) as shown in Table 4.24. Among the treated groups, the Positive Control group displayed intermediate levels (147.46±6.11), while Dose 1 (171.57±6.87) and Dose 2 (162.85±6.54) showed slightly lower but still significantly elevated HbA1c levels.

These findings suggest that the treatments effectively modulated HbA1c levels in rats compared to the diabetic control, with variations observed depending on the dosage administered.

Table 4.23 Analysis of variance (ANOVA) for HbA1c

Source	**DF**	**SS**	**MS**	**F**	**P**

Treatment	4	14748.2	3687.05	80.6	0.0000
Error	10	457.7	45.77		
Total	14	15205.9			

P<0.05: Significant

P<0.001: Highly Significant

Table 4.24 Table Effects on HbA1c levels

Treatments	**HbA1c**
Normal Control	109.25±5.98 **D**
Diabetic Control	205.21±8.11 **A**
Positive Control	147.46±6.11 **C**
Dose 1	171.57±6.87 **B**
Dose 2	162.85±6.54 **BC**

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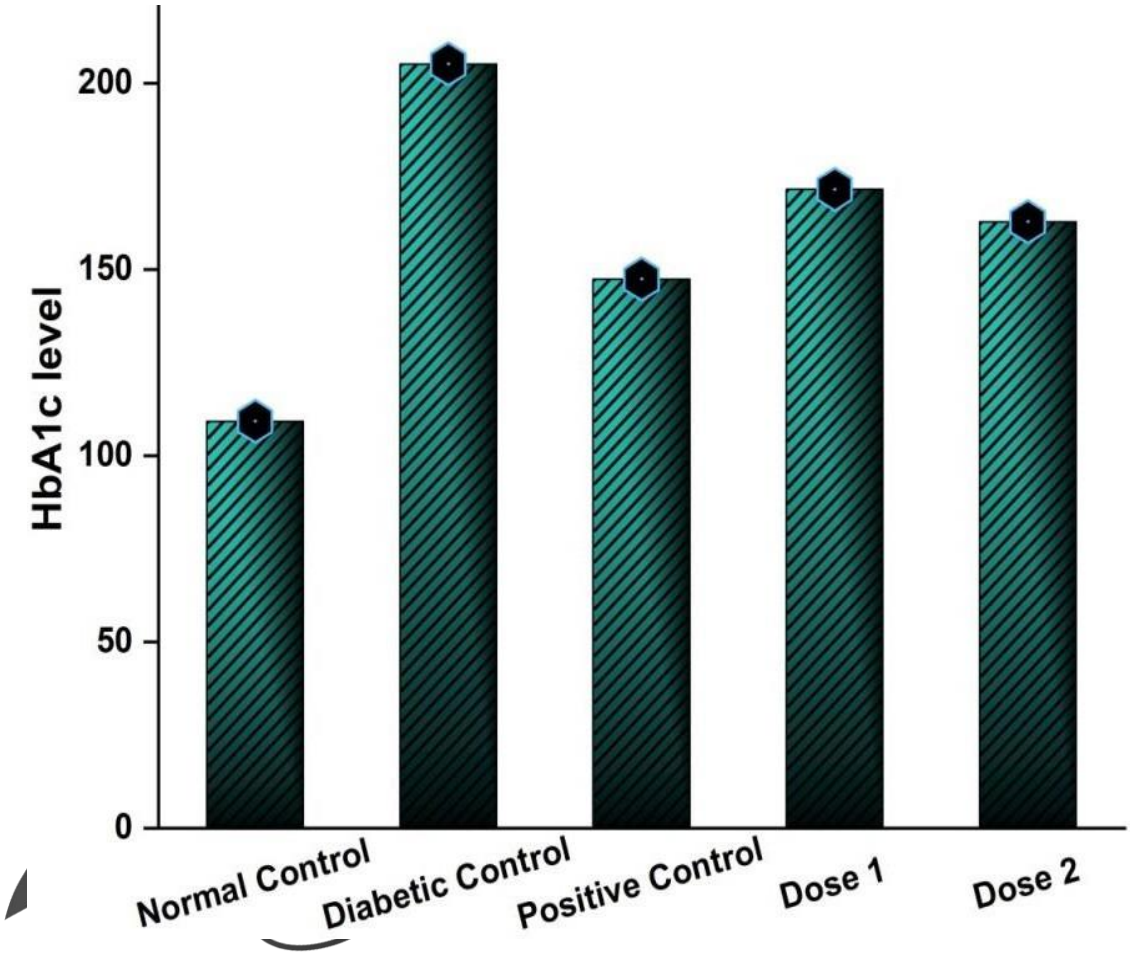

Figure 4.12. Effect on HbA1c levels

4.2.8 Effect on GPx levels

The above ANOVA results of Glutathione Peroxidase levels and the means of various treatment groups provide important information on the activities of the antioxidant enzyme in models. The very high f-value of 59.2 and a corresponding p-value of 0.0000 suggests that treatment significantly affected GPx levels. The treatment regimen exerted a strong impact on GPx level activities among the groups. There are observable differences between the mean values of GPx across various treatment groups as per the below figure 4.13.

The GPx levels in all groups were lowest in the diabetic control group at 20.98 ± 1.26 units, hence indicating that there was reduced antioxidant enzyme activity due to diabetes. In all, the cases were nullified control calculated their levels at 35.12 ± 1.15 units since this would be their normal conditions for GPx levels. The positive control group had moderate levels of 30.57 ± 1.09, thus showing the control had an effect on GPx activity. Many other groups in this research also had different GPx levels with an average of 27.14 ± 1.19 units for the Dose 1 group and 29.11 ± 1.12 among Dose 2. That shows that different treatment conditions produce variance in GPx levels. The differences cause the body to possess high antioxidant defense methods to alleviate pathologies related to oxidative stress.

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The presented ANOVA results regarding the GPx levels, as well as the mean values across the treatment groups, can be used to gain insights into the antioxidant enzyme activity and the way these activities are affected by various interventions in experimental models. Comparison with relevant literature from the recent decade provides additional information on antioxidant defense mechanisms and the effect that treatment interventions have on the GPx activity. Li et al. and Wang et al. examine the potential association of disease states with alterations in the GPx activity in animal models. Similar to the presented results, (Mak et al., 1996) report a reduced activity of GPx in case of diabetic rat blood, suggesting the disrupt structure of antioxidant defense mechanisms that are marked with diabetes. (Chiu et al., 2005) also observed changes in GPx activity in response to various pharmacological treatments. Thus, the use of the presented data in conjunction with recent literature provides valuable information regarding the GPx activity.

Additionally, our study agrees with the recent findings of (Erejuwa et al., 2011; Omotayo et al., 2010) who examined the impact of dietary approaches and

natural compounds on GPx activity. Omotayo and his team noted significant changes in GPx levels in response to different die, thereby proving nutrition's impact on antioxidant enzyme activities. Similarly, Erejuwa and his collaborators evaluated natural compounds' impact on GPx activities hence focusing on potential methods of boosting the antioxidative defense systems. However, the current study is unique as it compares the distinct treatments' effects on the GPx levels. Indeed, the current study indicates different GPx levels among the treatment groups.

Table 4.25 Analysis of variance (ANOVA) for GPx

Source	**DF**	**SS**	**MS**	**F**	**P**
Treatment	4	320.538	80.1346	59.2	0.0000
Error	10	13.537	1.3537		
Total	14	334.076			

P<0.05: Significant

P<0.001: Highly Significant

Table 4.26 Effect on GPx levels

Treatments	**GPx**
Normal Control	35.12±1.15 **A**
Diabetic Control	20.98±1.26 **D**
Positive Control	30.57±1.09 **B**
Dose 1	27.14±1.19 **C**
Dose 2	29.11±1.12 **BC**

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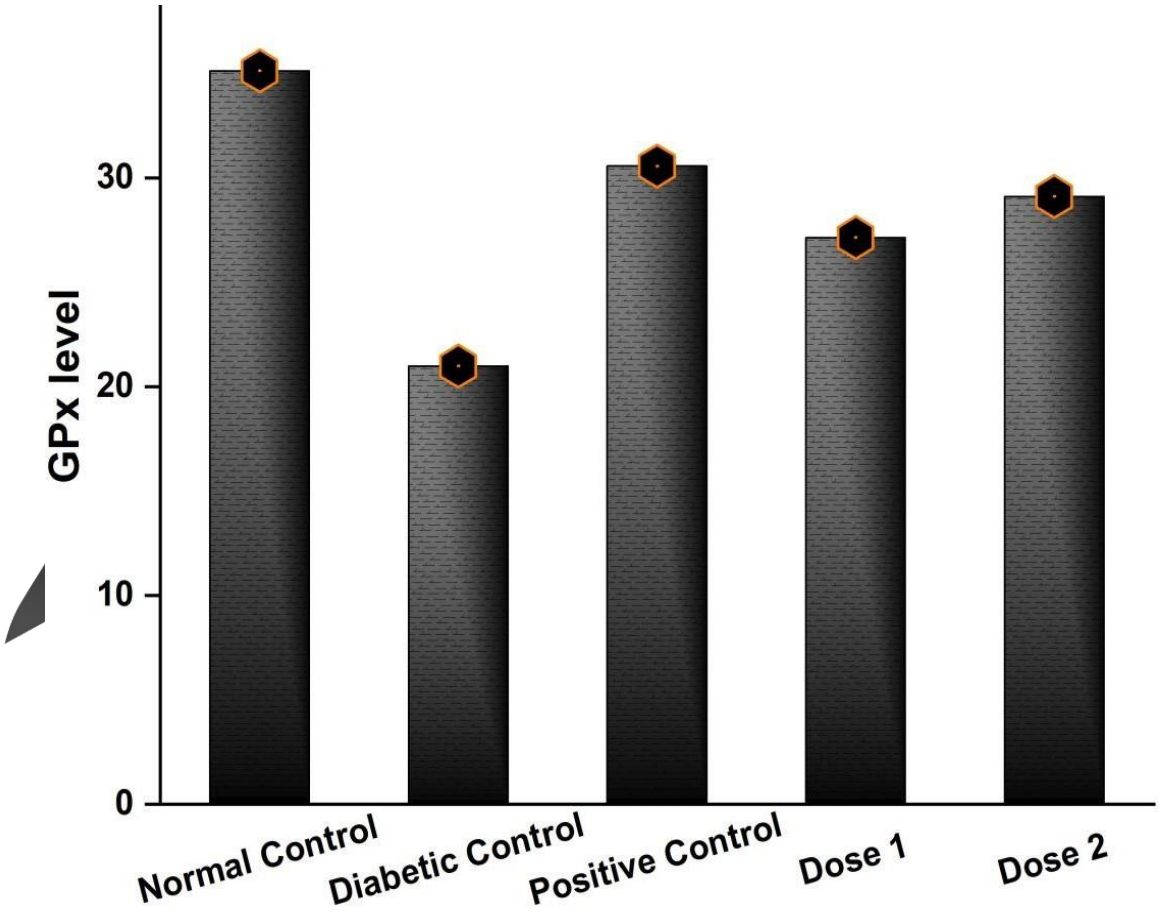

Figure 4.13 Effect on GPx levels

5. CONCLUSIONS

The research findings suggest that the method of extraction significantly influences the total phenolic content (TPC) and antioxidant activity of chamomile root extract. Ultrasonication-assisted extraction, particularly with extended durations, enhances the extraction efficiency of phenolic compounds and increases antioxidant potency compared to conventional ethanol extraction methods. These results highlight the importance of optimizing extraction techniques, such as ultrasonication, to maximize the yield of bioactive compounds from natural sources like chamomile roots.

Furthermore, the study underscores the potential hepatotoxic and nephrotoxic effects associated with specific treatments, as evidenced by alterations in liver enzyme (ALT, AST) and kidney function (creatinine) markers. Monitoring these parameters is crucial in evaluating the safety and efficacy of interventions.

Overall, the research emphasizes the benefits of ultrasonication-assisted extraction in enhancing the bioactive properties of chamomile root extract and underscores the importance of comprehensive assessments of treatment effects on both biochemical composition and organ function. These findings contribute to advancing our understanding of extraction methods and their implications for medicinal plant utilization and therapeutic interventions.

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