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Effect of Wheat Bread Incorporated with Chamomile and Wild Thyme on Glycemic Response, Antioxidant Status and Inflammation in Type 2 Diabetic Individuals

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ABSTRACT: Previous studies suggest that bread incorporated with chamomile and wild thyme powder exhibits lower starch digestibility and higher antioxidant activity *in vitro*; hence, its intake may suppress postprandial blood glucose and enhance antioxidant status in humans. The present study determined the effect of bread incorporating chamomile and wild thyme on blood glucose, antioxidant status, and inflammation in individuals with type 2 diabetes. Sixteen male subjects consumed either control bread (CB) or bread incorporating 3 % chamomile and wild thyme powder in two separate sessions. Blood glucose was measured using the finger-prick method at fasting and then postprandially for 2 h. Antioxidant and inflammatory markers were measured at fasting and 2 h after consumption. A non-significant decrease in blood glucose (–8.30 ± 2.39 mg/dl; p = 0.411) was observed after the consumption of chamomile- and wild thyme-containing bread (CWB) compared to CB. Compared to baseline, the consumption of CWB non-significantly increased total polyphenols, total antioxidant capacity (TOAC), and superoxide dismutase (SOD) activity by 6.12%, 20.32%, and 15.17%, respectively (p for all <0.05). Similarly, CWB non-significantly decreased thiobarbituric acid reactive substances (TBARS) by 27.46% and C-reactive protein (CRP) by 12.41% compared to baseline values (p for all <0.05). In conclusion, the consumption of bread incorporated with chamomile and wild thyme resulted in an improvement in blood glucose, an increase in polyphenols, TOAC, and SOD activity, and a reduction in TBARS and CRP levels in individuals with type 2 diabetes. Further studies are now warranted with larger sample sizes and higher doses to achieve significant clinical results.

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1. INTRODUCTION

Diabetes mellitus is the fourth most prevalent non-communicable disease globally, characterized by a deficiency in insulin secretion, insulin action, or both, resulting in hyperglycemia (Dilworth et al., 2021). Elevated blood glucose levels affect several organs and tissues in the body, increasing morbidity and mortality (Dhatariya et al., 2020). Long-term hyperglycemia elevates free radical generation and oxidative stress, resulting in protein glycation and membrane lipid peroxidation, thereby reducing the body's endogenous antioxidant defense system (Galicia-Garcia et al., 2020). Oxidative stress and inflammation also play a major role in the pathogenesis of cardiovascular diseases (Sardu et al., 2020). Over-inflammation in patients with type 2 diabetes mellitus leads to increased expression of the sodium-glucose cotransporter 2 (SGLT2), which in turn contributes to hyperglycemia and promotes the destabilization and rupture of atherosclerotic plaques (Sardu et al., 2021a). In recent years, attention has increasingly focused on non-traditional cardiovascular disease risk factors, such as excessive body fat accumulation, which contribute to chronic low-grade inflammation and cardiometabolic dysfunction in the general population (Sardu et al., 2018). Notably, women may exhibit sex-specific patterns of fat accumulation, including variations in breast fat content and density, that could negatively affect clinical outcomes by promoting systemic over-inflammation and down-regulation of sirtuins (Sardu et al., 2021b; Sardu et al., 2023).

Both pharmacological and non-pharmacological approaches are used to treat hyperglycemia. The most important non-pharmacological therapeutic techniques for diabetes control are medical nutrition therapy and exercise. Pharmacological options include oral antihyperglycemic medications, synthetic drug combinations, insulin therapy, phytoconstituents, and the combination of herbal phytoconstituents with synthetic therapies (Khursheed et al., 2019). Dietary fiber, antioxidants, polyphenols, glucosinolates, inducers of beneficial enzyme activities, polyunsaturated fatty acids, prebiotics, and probiotics are examples of herbal phytoconstituents that may reduce hyperglycemia (Forni et al., 2019).

Chamomile (*Matricaria chamomilla* L.) is a medicinal herb that contains over 120 bioactive chemicals and has been traditionally used to alleviate intermittent fevers for centuries (Singh et al., 2011). In animal studies, chamomile extract ameliorated hyperglycemia and HbA1c levels (Emam, 2012; Rajagopalan et al., 2016), enhanced insulin levels and insulin sensitivity (Abdolmaleki & Heidarianpour, 2018), and preserved the insulin-producing cells of pancreatic islets (Cemek et al., 2008) in the treatment group compared to the placebo group. In human studies, chamomile tea showed a marked decrease in HbA1c concentration, serum insulin levels, inflammatory markers, and oxidative stress markers, as well as an improvement in the lipid profile and antioxidant markers (Hajizadeh et al., 2020). The antihyperglycemic mechanisms of chamomile are attributed to the modulation of peroxisome proliferator-activated receptors (PPARs), inhibition of intestinal α-glucosidase activities, inhibition of liver glycogen phosphorylase, which enhances hepatic glycogen content, and inhibition of intestinal glucose transport, causing a decrease in carbohydrate absorption (Kato et al., 2008).

Wild thyme (*Thymus serpyllum* L.), another medicinal herb, is used for various purposes, such as an expectorant, anthelmintic, antiseptic, carminative, sedative, and tonic (Jaric et al., 2015). In previous animal studies, wild thyme was shown to lower blood glucose levels and improve glucose and insulin tolerance in diabetic animal models (Azhar et al., 2022; Alamgeer et al., 2012). The possible mechanisms underlying the beneficial effects of wild thyme in lowering blood glucose include the inhibition of α-amylase activity and improvement in insulin sensitivity (Wahab et al., 2022). These findings support the use of wild thyme as a beneficial supplement for diabetes. However, despite the beneficial effects of wild thyme in modulating blood glucose in animal models, its potential benefits for type 2 diabetes in humans have not yet been reported.

White bread is a high-glycemic staple food that contains a high amount of rapidly digested starch, which promptly increases postprandial blood glucose and is, therefore, not recommended for individuals with diabetes (Amoah et al., 2022). Therefore, modulating the glycemic features of bread may be a key strategy to allow its consumption by individuals with diabetes. In this context, different plant-based functional ingredients, such as herbs, underutilized cereal grains, seeds, and other plant parts, have been incorporated into bread because of their health-promoting effects, including, but not limited to, reducing the glycemic index of bread and enhancing its nutritive value, bioactive properties, and antioxidant activity (Schadow et al., 2023; Amini et al., 2020).

To our knowledge, no study has yet explored the effects of consuming bread containing chamomile and wild thyme on the metabolic profile of individuals with type 2 diabetes. Therefore, the present study aimed to investigate the effect of this modified bread on postprandial glycemia, antioxidant status, and inflammation in individuals with type 2 diabetes. We hypothesized that the consumption of the modified bread would significantly improve the post-meal metabolic parameters related to glucose metabolism and antioxidant status in individuals with type 2 diabetes compared to standard control bread.

2. MATERIALS AND METHODS

2.1. Procurement of raw materials

All-purpose wheat flour (Waqas General Flour Mills, Pakistan) and other baking materials, including salt, sugar, butter, and yeast, were procured from the local market. Wild thyme was procured from authorized dealers in Skardu, Gilgit-Baltistan, while chamomile was purchased from authorized dealers in Karachi (Pakistan's First Herbal Store, Karachi) in sealed plastic containers. The samples were powdered using a commercial grinder and were stored in plastic jars until use.

2.2. Test breads

The bread was prepared by the straight dough method. Briefly, a basic dough recipe on a 100 g flour basis for control bread (CB) was followed by mixing the flour with weighed dry ingredients. The dough was prepared by adding 6 g sugar, 1 g salt, 2 g yeast, 5 g oil, and 60 ml water to 100 g all-purpose wheat flour. For the preparation of chamomile- and wild thyme-containing bread (CWB), the chamomile and wild thyme powders were first mixed in equal proportions. Wheat flour was then replaced with the mixture at a 3% incorporation level. This level of substitution was chosen based on a previous consumer acceptability study conducted in our laboratory (Abbas et al., 2024). All other ingredients were kept at the same level for the preparation of the treatment bread. The dough was kneaded by hand for 40 min at room temperature and transferred into baking pans for fermentation for 30 min. The bread was baked for 20 min at 220 °C in an electric baking oven (Panasonic Digital Oven).

2.3. Subject recruitment

Sixteen male individuals with diabetes were recruited through personal contacts. The inclusion criteria were: age 30–60 years, BMI ≥ 25.0 kg/m^2, and fasting blood glucose > 6.1 mmol/L. The study exclusion criteria were: use of insulin, history of gastrointestinal disease, food allergies, smoking, and breakfast skipping. A health screening questionnaire was used for screening. Full details of the study protocol were explained to the participants, and their queries were answered before study initiation. They were also allowed to withdraw from the study at any time. This study was performed according to the guidelines laid down in the Declaration of Helsinki. The study was approved by the Human Research Ethics Committee of the Department of Human Nutrition (HN-HREC/2020-008), The University of Agriculture, Peshawar. All the subjects provided written informed consent to participate in the study.

2.4. Study design and protocol

Subjects were instructed to follow their usual diet and avoid strenuous physical activity during the study period. Each subject attended two testing sessions. At each session, subjects consumed one of the two test breads in a random order. Upon arrival at the laboratory after an overnight fast, baseline measurements, including height, weight, and waist circumference, were obtained. After 5 min of rest, a peripheral venous catheter was inserted into an antecubital vein. Fasting blood samples were collected in EDTA tubes by venipuncture for the analysis of insulin and other parameters. Blood glucose was measured in capillary blood using the finger-prick method. Afterward, the test bread was served to the subjects with water (250 ml) as breakfast. The portion size of the bread was adjusted to provide 50 g available carbohydrates. The composition of the test breakfast meal is shown in Table 1. Subjects were requested to finish the test bread within 10 min and were advised not to consume anything during the next 2 h. Venous blood samples were again collected from the subjects at 2 h after consuming the bread. This time was selected based on the peak absorption time (90–150 min) of polyphenols reported in the literature (Torabian et al., 2009). Plasma was separated from whole blood by centrifugation (3000 rpm for 15 min) at 8 °C. The plasma was aliquoted and stored in cryovials at –80 °C until analysis.

2.5. Blood glucose analysis

Blood samples from the subjects were collected from the warmed fingertip using a lancet device, following glycemic

Table 1
Composition of control and test breads.

Ingredients	CB	CWB
Bread (g)	103	104
Energy (Kcal)	240	248
Av. CHO (g)	50	50
Protein (g)	7.88	8.98
Fats (g)	1.02	1.48
Dietary fiber (g)	0.584	2.15
Total polyhenols	36.08	84.14

Av. CHO: available carbohydrates; CB: control bread; CWB: chamomile and wild thyme-containing bread.

index testing protocols (Brouns et al., 2005). Briefly, the subjects had their fingers warmed before the prick to increase blood flow. Capillary blood samples were collected without squeezing the finger (to avoid dilution of plasma). Blood glucose levels were measured at baseline (immediately before bread consumption) and thereafter at 30, 45, 60, 90, and 120 min using an Accu Check glucometer. The first drop of blood was discarded to avoid contamination, and the second drop was applied onto the glucose testing strip. The same glucometer was used throughout the study to minimize intra-subject variation.

2.6. Measurement of plasma total polyphenols

Total polyphenols were first extracted from the blood plasma as previously described (Khan et al., 2015). The Folin-Ciocalteu method adopted by Hajira & Khan (2022) was then used for the determination of plasma total polyphenols. Briefly, 100 µl of the concentrated extract was mixed with 500 µl of 0.2 N Folin-Ciocalteu reagent and 400 µl of Na_2CO_3 (2 M) solution. The mixture was incubated for 90 min at 25 °C in the dark. The absorbance was measured using a microplate reader (Multiskan Go, Thermo Fisher Scientific, USA) at 750 nm. Milli-Q water (100 µl) was used as the blank. A calibration curve was constructed using gallic acid concentrations in the range of 0–500 mg/l, and the results were presented as mg of gallic acid equivalents (GAE) per liter of blood.

2.7. Total antioxidant capacity (TAOC)

TOAC was measured using an enzyme-linked immunosorbent assay (ELISA) kit: Cat. No. E2199Hu (Bioassay Technology Laboratory, China). This assay is used for the accurate quantitative detection of human total antioxidant capacity (TOAC) in serum, plasma, cell culture supernates, cell lysates, and tissue homogenates. Its calibration curve ranges from 0.3 U/ml-90 U/ml, with a sensitivity of 0.14 U/ml. All reagents, standard solutions, and samples were prepared as per the manufacturer's instructions. The assay was performed at room temperature. Briefly, 50 µl of standard was added to the standard well, and 40 µl of sample was added to the sample wells. Antibody was not added to the standard well because the standard solution already contained the required biotinylated antibody. To the sample wells, 10 µl of anti-TAOC antibody was added. Then, 50 µl of streptavidin-HRP was added to the sample and standard wells (but not the blank control well). The samples were mixed thoroughly, covered with a sealer, and incubated for 60 min at 37 °C. The sealer was then removed, and the plate was washed five times for 1 min with wash buffer (0.35 ml). After blotting onto a paper towel, 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well. The plate was then covered with a new sealer and incubated for 10 min at 37 °C in the dark. Next, 50 µl of stop solution was added to each well, and the optical density was determined within 10 min at 450 nm using a microplate reader (Multiskan Go, Thermo Fisher Scientific, USA). A standard curve was constructed to measure the TOAC.

2.8. Superoxide dismutase (SOD) activity

SOD activity was determined by ELISA. A SOD-1 assay kit (Cat. No. E0700 Hu) was purchased from Bioassay Technology Laboratory, China. The plate was pre-coated with a human SOD-1 antibody. Samples were added to the wells and bound to the coated antibodies. Then, biotinylated human SOD-1 antibodies were added, which conjugated with the SOD-1 in the samples. Afterward, streptavidin-HRP was added and combined with the biotinylated SOD-1 antibody. After incubation, unbound streptavidin-HRP was washed away during the washing step. Then, the substrate solution was added, and color developed in proportion to the amount of human SOD-1. The reaction was terminated by the addition of an acidic stop solution, and the absorbance was measured at 450 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific, USA). The concentration was determined using the calibration curve.

2.9. Thiobarbituric acid reactive substance (TBARS)

TBARS was determined using an ELISA kit: Cat. No. E3642 Hu (Bioassay Technology Laboratory, China). This kit had a standard curve range of 0.05 nmol/ml-30 nmol/ml, with a sensitivity of 0.022 nmol/ml. A 50 µl standard containing biotinylated antibody was added to the standard well. Next, 40 µl of sample was added to the sample wells along with 10 µl of anti-TBARS antibody. Then, 50 µl of streptavidin-HRP was added to both the sample and standard wells. The plate was then covered with a sealer and incubated for 60 min at 37 °C. The sealer was removed, and the plate was washed five times with wash buffer. After that, 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well. The plate was covered with a new sealer and incubated for 10 min at 37 °C in the dark. Then, 50 µl of stop solution was added to each well, and the optical density was measured at 450 nm using a Multiskan GO microplate reader (Thermo Fisher Scientific, USA). A calibration curve was constructed for measuring the TBARS concentration.

2.10. High sensitivity C-reactive protein (hs-CRP)

Plasma hs-CRP was measured using a high-sensitivity immunoturbidimetric assay with a Roche Diagnostics kit (Roche Diagnostics, Ireland), according to the manufacturer's instructions. The assay was performed using a Roche/Hitachi automated analyzer (Cobas C311, Roche Diagnostics GmbH, Mannheim, Germany). The method is based on the agglutination of CRP with latex particles coated with monoclonal anti-CRP antibodies. The intensity of the resulting turbidity is directly proportional to the concentration of hs-CRP in the sample and was measured at 546 nm. Results were obtained automatically using six levels of a calibration curve and reported as mg hs-CRP per dl. The inter-assay coefficient of variation was < 6%.

2.11. Statistical analysis

SPSS (version 21, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Normality of the data and homogeneity of variance were checked using the Kolmogorov–Smirnov and Levene's tests, respectively. Values are presented as means ± SEMs. Two-way repeated-measures ANOVA with *post hoc* Bonferroni adjustment was used to investigate the effects of time, treatment, and their interaction on blood glucose. The effect of treatment on blood glucose iAUCs was determined using a paired t-test. For intragroup comparisons (baseline vs. 2-hour values) of plasma polyphenols, antioxidant markers (TAOC, SOD activity, and TBARS), and hs-CRP, a paired-samples t-test was used. A $p < 0.05$ was considered statistically significant.

3.RESULTS

3.1. Baseline characteristics

Baseline data of the diabetic individuals are presented in Table 2. The mean age, body mass index, fasting blood glucose, and waist circumference of the subjects were 49.10 years, 27.06 kg/m^2, 205.75 mg/dl, and 99.2 cm, respectively.

3.2. Blood glucose response in diabetic subjects

The mean change in blood glucose from baseline and the incremental areas under the curves (iAUC) in diabetic subjects after the consumption of 3% CWB or CB are presented in Figure 1. A significant effect of time ($p < 0.001$) but non-significant effects of treatment ($p = 0.411$) and time × treatment ($p = 0.813$) on postprandial blood glucose levels were observed. The consumption of CWB resulted in a lower blood glucose concentration compared to CB. However, this difference was not significant. The highest mean difference in peak blood glucose concentration between CWB and CB, of 8.30 mg/dl, was observed at 60 min. There was no significant difference ($p = 0.367$) in iAUC between CWB and CB.

3.3 Plasma total polyphenols

The total polyphenol levels of the subjects at baseline and after 2 h of consumption of the treatment breads are shown in Figure 2. The total polyphenol content in plasma slightly decreased at 120 min compared to the baseline values (0 min) after the consumption of CB. The plasma polyphenol content increased by 6.12% at 120 min after the consumption of CWB compared to the baseline values. However, the increase was not significant ($p > 0.05$).

3.4 Plasma total antioxidant capacity

The plasma total antioxidant capacity (TOAC) of both CWB and CB is shown in Figure 3. TOAC improved, and an increase of 20.32% was observed after the consumption of CWB at 120 min compared to the baseline values (0 min). However, this increase was not statistically significant ($p > 0.05$).

3.5. Plasma superoxide dismutase (SOD) activity

The plasma SOD activity levels are presented in Figure 4. No significant changes were observed in SOD activity after

Table 2
Baseline characteristics of the Diabetic participants (n = 16)*

Variable	Value
M/F (n)	16/0
Age (y)	49.10 ± 1.62
Weight (kg)	78.44 ± 2.99
Height (m)	170.20 ± 1.89
BMI (kg/m^2)	27.06 ± 1.04
Waist circumference (cm)	99.20 ± 2.30
Fasting plasma glucose (mg/dl)	205.75 ± 13.31
Systolic blood pressure (mm Hg)	132.60 ± 5.03
Diastolic blood pressure (mm Hg)	95.50 ± 2.84

*Values are means ± SD.

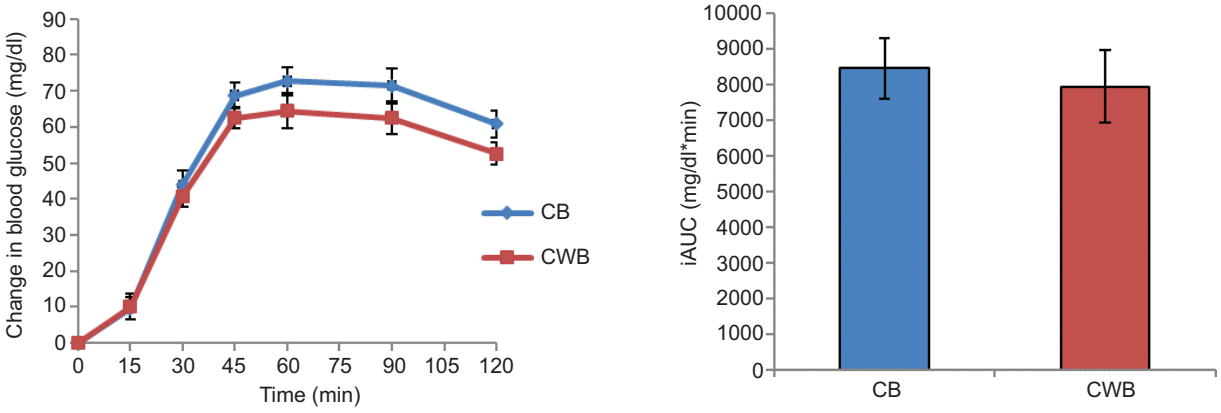

Figure 1. Mean (± SEM) changes from baseline in blood glucose (n = 16) and incremental areas under the curves (iAUC) in diabetic subjects after the consumption of test breads. CB: control bread; CWB: chamomile- and wild thyme-containing bread. Values were not significantly different at each time point (two-way repeated-measures ANOVA with Bonferroni adjustment). Vertical bars were not significantly different (paired t-test).

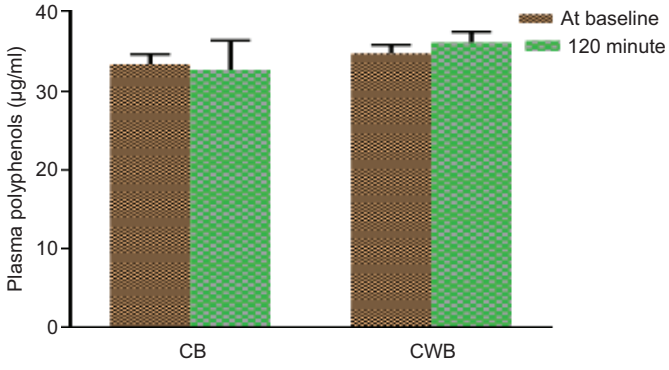

Figure 2. Plasma total polyphenol levels (mean ± SEM) in diabetic subjects (n = 16). CB: control bread; CWB: chamomile- and wild thyme-containing bread. Vertical bars within each group were not significantly different (paired t-test).

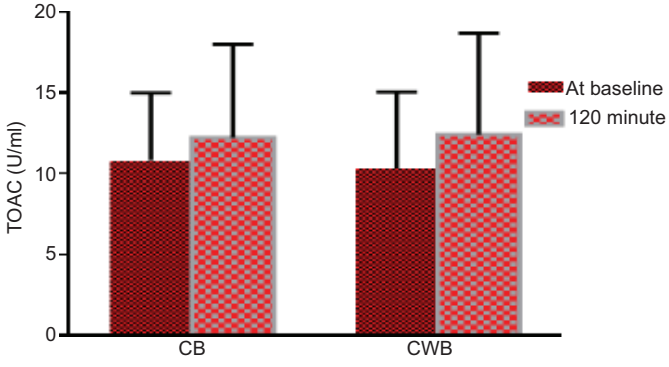

Figure 3. Plasma total antioxidant capacity (mean ± SEM) in diabetic subjects (n = 16). CB: control bread; CWB: chamomile- and wild thyme-containing bread. Vertical bars within each group were not significantly different (paired t-test).

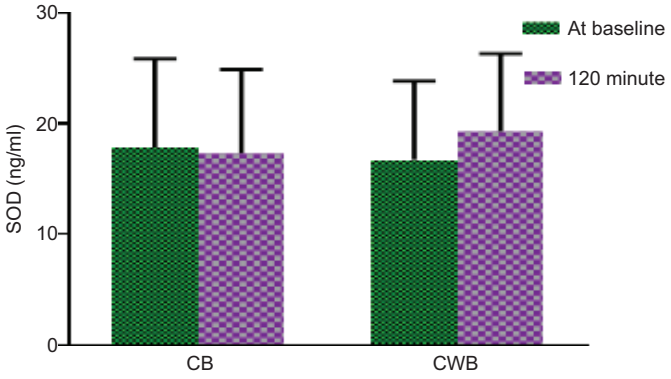

Figure 4. Plasma superoxide dismutase activity (mean ± SEM) in diabetic subjects (n = 16). CB: control bread; CWB: chamomile- and wild thyme-containing bread. Vertical bars within each group were not significantly different (paired t-test).

the consumption of either CB or CWB at 120 min compared to the baseline values (0 min). CWB resulted in a 15.17% increase in SOD activity at 120 min compared to the baseline values (0 min); however, this increase was not significant ($p > 0.05$).

3.6. Plasma thiobarbituric acid reactive substances

The concentration of TBARS is presented in Figure 5. At baseline, TBARS levels in both groups were almost the same. Plasma TBARS levels did not differ significantly ($p > 0.05$) at 2 h after the consumption of either of the two test breads compared to their corresponding baseline values (0 min).

A 27.46% decrease was observed in TBARS at 120 min compared to the baseline values (0 min) after the consumption of CWB; however, this decrease was not significant ($p > 0.05$).

3.7. High-sensitivity C-reactive protein

Serum hs-CRP is presented in Figure 6. The mean hs-CRP concentration of CB at fasting was 1.6 ± 2.25 mg/l. This level remained almost stable (1.85 mg/l) at 120 min after the consumption of CB. The hs-CRP level decreased by 12.41% at 120 min after the consumption of CWB compared to the baseline values (0 min); however, these changes were not statistically significant ($p > 0.05$).

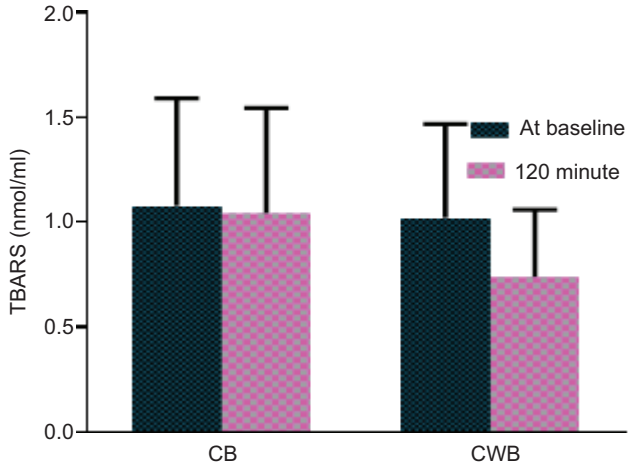

Figure 5. Plasma thiobarbituric acid reactive substances levels (mean ± SEM) in diabetic subjects (n = 16). CB: control bread; CWB: chamomile- and wild thyme-containing bread. Vertical bars within each group were not significantly different (paired t-test).

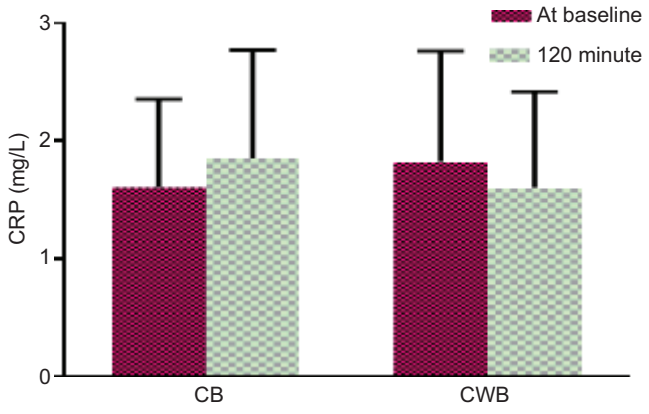

Figure 6. High-sensitivity C-reactive protein levels (mean ± SEM) in diabetic subjects (n = 16). CB: control bread; CWB: chamomile- and wild thyme-containing bread. Vertical bars within each group were not significantly different (paired t-test).

4. DISCUSSION

The current study, for the first time, investigated the effect of CWB on the glycemic profile and antioxidant status in a cohort of individuals with diabetes. Consumption of CWB resulted in an improvement in the glycemic response. It also enhanced plasma polyphenol levels, antioxidant capacity, and SOD activity and decreased markers of oxidative stress (TBARS) and inflammation (hs-CRP).

The results of the current clinical trial regarding the antihyperglycemic potential of chamomile were consistent with previous studies. Cemek et al. (2008) and Emam (2012) demonstrated that chamomile extracts significantly reduced blood glucose levels and improved insulin sensitivity in streptozotocin (STZ)-induced diabetic rats. Polyphenols found in chamomile have been shown to dramatically decrease circulating blood glucose levels in diabetic rats by inhibiting intestinal alpha-glucosidase activities (Alam et al., 2022), reducing oxidative stress (Cemek et al., 2008), upregulating peroxisome proliferator-activated receptors (PPARs) (Weidner et al., 2013), modulating glucose transporter (GLUT-4) receptors, and triggering the 5-adenosine monophosphate protein kinase (AMPK) pathway by enhancing the expression of the adiponectin gene (Ding et al., 2010).

The current study's findings on the antihyperglycemic properties of wild thyme (WT) were similarly consistent with earlier investigations. In their review, Jalil et al. (2024) pointed out that the alkaloids, glycosides, thymol or carvacrole, flavonoids, and terpenoids present in wild thyme reduced blood glucose levels, mimicked insulin, decreased glucagon secretion, restored impaired β-cells, promoted glycolysis, and decreased glucose absorption from the alimentary canal through the inhibitory action on α-glucosidase release.

The findings of the current study regarding plasma polyphenol levels and antioxidant capacity were consistent with those of previous research. Dietary and plasma polyphenol contents were investigated by several researchers using different foods separately or by incorporating one or more food ingredients. Fuhrman et al. (2005) used fruits, while tea, olive oil, chocolate, and red sorghum were used by Henning et al. (2004), Covas et al. (2003), Serafini et al. (2003), and Khan et al. (2015), respectively, in separate studies. They reported a direct relationship between polyphenols and total antioxidant capacity and suggested that the consumption of a polyphenol-rich diet (1-2 servings/day) increases both plasma polyphenol levels and plasma antioxidant capacity. Although they could not explain the exact mechanisms or

absorption sites of polyphenols in humans, they speculated that the absorption of polyphenols might have occurred mostly within 90 min in the upper gastrointestinal tract. However, they hypothesized that polyphenols present in the diet reduced the production of superoxide anions, lipid peroxyl radicals, and hydroxyl radicals through their antioxidant properties, thereby improving cardiometabolic health. These authors suggested further studies to fully elucidate the kinetics of polyphenols in humans while measuring some of the urinary metabolites.

Oxidative stress is a toxic process that injures the body's cells. Premature aging, arthritis, cardiovascular diseases, diabetes, cancer, autoimmune disorders, and neurological disorders are all associated with increased oxidative stress (Gupta et al., 2014). The current study's findings were also consistent with those of Ramadan and Emam (2012). They treated diabetic animals with an aqueous extract of chamomile (100 mg/kg/day) and observed that the levels of endogenous antioxidant enzymes increased significantly, bringing oxidative stress closer to normal levels. Compared to untreated diabetic animals, this intervention considerably reduced malondialdehyde (MDA) production. The same results were confirmed by Alouie et al. (2017) and Al-Musa & Al-Hashem (2014) in other animal studies. Similarly, in a human study, Zemestani et al. (2016) found that consuming three cups of chamomile tea daily (3 g/150 ml hot water) for eight weeks significantly increased SOD, catalase, and glutathione peroxidase activities, as well as significantly reduced MDA levels in type 2 diabetic individuals.

The consumption of CWB decreased hs-CRP by 12% compared to the baseline values in the present study. Several researchers have investigated the effects of chamomile extract and essential oil on inflammation (Wu et al., 2012; Fabian et al., 2011). They concluded that chamomile extract affected the vascular system by reducing inflammation and edema caused by croton oil in the ears and paws of rats, with effects similar to those of benzydamine. Zemestani et al. (2018) determined the anti-inflammatory impact of consuming 3 g/day of chamomile tea in individuals with type 2 diabetes and observed a reduction in tumor necrosis factor-α (TNF-α) and high-sensitivity C-reactive protein (hs-CRP). Although the present study showed a decrease in oxidative stress and inflammatory markers, these effects were not significant. The possible reason for the lack of significant effects on these biomarkers could be attributed to the low dose and the acute nature of the current study.

The key strengths of the current study include its well-designed randomized controlled crossover trial and its novelty as the first to determine the effect of bread incorporated with chamomile and wild thyme on glycemic response and markers of oxidative stress in individuals with type 2 diabetes. The current study has some limitations, including a small sample size, its acute nature, a low dose of chamomile and wild thyme, and only a single postprandial blood collection. Therefore, future studies with larger sample sizes, longer durations, higher doses, and repeated postprandial blood collections are recommended. In addition, other endogenous antioxidant enzymes and oxidative stress markers should also be investigated. The study included only male participants; therefore, future research should include both sexes to enhance the generalizability of the findings.

5. CONCLUSION

In conclusion, 3% CWB non-significantly improved postprandial blood glucose, plasma polyphenol levels, antioxidant capacity, and SOD activity in individuals with type 2 diabetes. Similarly, a non-significant reduction in the biomarkers of lipid peroxidation (TBARS) and inflammation (CRP) was observed. In future studies, the duration and dose of chamomile and/or wild thyme should be considered to achieve an inhibitory influence on carbohydrate and lipid absorption.

FUNDING

This study received no financial support.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

I.K. and I.A. designed the study. I.K. and I.A. conducted the study and analyzed the data. I.K., I.A., and J.A. drafted the manuscript. I.K., M.I.W., A.S.R.A., and S.K.J. critically revised the manuscript. All authors read and approved the final version of the manuscript.

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