



## RESEARCH ARTICLE

### Exploring the Therapeutic Potential of *Matricaria Chamomilla* and *Hibiscus Rosa-Sinensis* Against Diabetes Mellitus

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#### ABSTRACT

Diabetes mellitus is a metabolic disease and a major global health concern, characterized by consistently elevated blood glucose levels. Although various treatments are available, they come with certain limitations and side effects. Consequently, herbal alternatives have gained interest for their potential effectiveness in managing diabetes. Chamomile and hibiscus are two herbs known for their health benefits. The study aimed to examine the effects of these herbs both individually, and in combination, on blood glucose levels. Four varieties of value-added peach drinks were developed by incorporating ethanol extracts of both herbs. *In vivo*, the administration of chamomile and hibiscus extracts-based drinks was carried out. The study was divided into six groups: G<sub>0</sub> (Negative control), G<sub>1</sub> (Positive control), G<sub>2</sub> (Chamomile added peach drink), G<sub>3</sub> (Hibiscus added peach drink), G<sub>4</sub> (Chamomile and Hibiscus added peach drink), and G<sub>5</sub> (Hyperglycemic rats+ metformin). It was observed that the treatments had a significant impact on all biomarkers by bringing them to normal range, particularly in the combined chamomile and hibiscus treated group. There was an increase in serum insulin and HDL levels along with a decrease in FBG, RBG, and other lipid profile markers such as LDL, TC, and, TG. Normal levels of liver and kidney biomarkers are used as indicators for assessing the safety of chamomile and hibiscus-based drinks. The results of the current study suggest that both chamomile and hibiscus have antidiabetic properties, and their combination had superior outcomes, comparable to the standard drug (metformin). Therefore, these herbs could serve as healthier and more accessible alternatives for managing diabetes, particularly in regions where conventional medicine may not be readily available.

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#### INTRODUCTION

Diabetes is one of the most common public health issues in the world. As a result of decreased insulin production or action, this condition of carbohydrate metabolism causes blood glucose levels to remain elevated for an extended period. It is consistently prevalent in Southeast Asia, with Pakistan standing out as a particularly alarming case. According to the International Diabetes Federation (IDF) 2021, the prevalence of diabetes in Pakistan among adults (ages 20–79), is estimated at 30.8%, making it the highest globally (Azeem *et al.*, 2022). In the year 2000, the estimated number of affected individuals was 171 million, and it is predicted to reach 552 million by 2030 and 693 million by

2045 across all age groups (Aljadani *et al.*, 2025). As per the International Diabetes Federation's data for 2022, in Pakistan approximately 26.7% of adults are affected by diabetes, resulting in an estimated total of around 33 million cases. The increasing prevalence of diabetes is concerning, as many cases may remain undiagnosed, potentially leading to an elevated risk of complications due to delayed treatment (Azeem *et al.*, 2022).

Various strategies have been developed for the treatment of diabetes, but they are costly and have side effects. For example, metformin is an antidiabetic drug that can cause side effects such as gas, diarrhea, and nausea (Do *et al.*, 2012). In contrast, herbal remedies are generally associated with fewer side effects compared to pharmaceutical medications (Farzaneh and Carvalho,

2015) and have been consumed for medicinal uses for thousands of years (Abdel Salam *et al.*, 2025). Although many herbs have traditionally been used in the management of diabetes but some are costly, so they are usually combined in smaller doses and allowed to act on multiple particular sites within the body separately or synergistically (Sotoudeh *et al.*, 2019).

Chamomile is a widely used medicinal plant belonging to the Asteraceae family (Mojibi *et al.*, 2022). In Pakistan, it is predominantly found in Balochistan, specifically in Mastung and Nushki, with a scattered distribution across Europe and temperate Asia. However, it is considered quite rare, being only spotted in the Mastung and Nushki regions of Balochistan and available in the market as "Gul-e-Baboona" (Ahmad *et al.*, 2009). Chamomile is being utilized in various commercial products, such as tea, infusions, pills, and liquid. The active components of chamomile are esculetin, herniarin, umbelliferone, coumarins, quercetin, luteolin, apigenin, flavonoids (Petronilho *et al.*, 2012). Chamomiles contain volatile and non-volatile active components that are responsible for their therapeutic properties (Singh *et al.*, 2011). Based on studies of both experimental and clinical reports, chamomile extracts and essential oils were found to have antidiabetic properties (Ivanovic *et al.*, 2014). Flower heads of German chamomile have been utilized in herbal teas and other preparations made from extracts. Chamomile extracts and their flower heads are used in various teas, herbal remedies, cosmetics, insect repellents, food flavors, and dyes (Sowjanya and Shubha, 2015).

*Hibiscus rosa-sinensis* is prevalent in cotton-growing regions of Pakistan and India, commonly used as an ornamental hedge plant (Akhtar *et al.*, 2014). It thrives in regions with tropical and subtropical climates, and can even be grown in controlled environments like glass chambers in colder climates, provided temperatures remain above 10°C. Different parts of this herb have also been evaluated for medicinal purposes against various diseases (Sağlam *et al.*, 2023). Hibiscus has been evaluated for its medicinal properties in treating various conditions, including diabetes, cardiovascular disease, inflammation, tumors, and diarrhea (Ghule *et al.*, 2015). Traditionally, it has been used in Northern Nigeria to relieve constipation, with its red calyx utilized in jellies, jams, and teas. In traditional medicine, hibiscus leaves serve as emollients and aperients for skin conditions and burning sensations (Khristi and Patel, 2016). The leaves and roots have emmenagogue and anodyne properties, promoting blood circulation and regulating menstruation. Hibiscus has also been used to induce abortion, stimulate the placenta after childbirth, and treat headaches, high blood pressure, and stomach pain. A decoction of its fruits, roots, and leaves is used for treating coughs, boils, and arthritis, while the fruits are applied externally to heal wounds and ulcers (Al-Snafi, 2018). It has been reported that *H. rosa-sinensis* flowers consist of saponins, alkaloids, steroids, flavonoids tannins, total proanthocyanidin, quercetin-3-diglucoside, cyaniding-3-soporoside-5-glucoside, cyaniding-3-5-diglucoside, vitamins, ascorbic acid, niacin, riboflavin and thiamine as chemical constituents. Moreover, four different flavonoids were identified in the flowers of hibiscus such as myricetin, kaempferol, quercetin, and rutin. Cyanidin 3-

soporoside was the most abundant anthocyanin in its flower (Sarwade *et al.*, 2025).

In a nutshell, diabetes is increasing day by day and various strategies have been developed for its treatment, but they are costly and have side effects. In this regard, the use of locally available, low-cost natural sources can reduce costs as well as side effects. Herbal plants have been proven to be beneficial for treating various diseases, and pharmaceutical companies use them to develop new medicines. Keeping in view the above-mentioned facts i.e., low cost, fewer side effects, and health benefits of *Matricaria chamomilla* and *Hibiscus rosa-sinensis*, the present research was conducted to develop a peach drink containing above mentioned herbal extracts. Subsequently, the focus of the investigation shifted towards conducting animal experimental modeling to assess the therapeutic capacity of the developed value-added peach drinks for diabetes.

## MATERIALS AND METHODS

**Procurement of raw materials:** The flowers of two herbs i.e., *Matricaria chamomilla* and *H. rosa-sinensis* were collected from the botanical garden UAF, Faisalabad, and Peach fruit was purchased at the Faisalabad local market. All of the chemicals and reagents used for the research were bought from (Sigma Aldrich, Tokyo, Japan) and (Merck KGaA, Darmstadt, Germany). Metformin was purchased from Sanofi-Aventis (Pvt.) Ltd., Pakistan. For the bio-efficacy trial, male (Sprague Dawley) rats were purchased from the National Institute of Health (NIH), Islamabad, and kept in the NIFSAT. UAF, Faisalabad animal facility, to conduct an effective investigation.

**Sample Preparation:** The flowers of chamomile and hibiscus were washed thoroughly and dried at 60°C for 24 hours, then ground into powder and stored in airtight containers in a cool, dry place (Prabawa *et al.*, 2023).

**Preparation of Extract:** Ethanolic extracts were prepared by placing 20 grams of each flower sample into a conical flask and diluting it with 250 mL of ethanol. The flasks were sealed with aluminum foil to prevent solvent evaporation and then placed on an orbital shaker set at 250 rpm for 8 hours. Afterward, the mixtures were filtered using Whatman filter paper No. 4. The solvent was then evaporated using a rotary evaporator at 40°C, and the extracts were further dried in a hot air oven (Anjum *et al.*, 2021; Ahmad *et al.*, 2025).

**Development of Value-Added Peach Drink:** The drinks were prepared using 250 mg/kg body weight (b.w.) of ethanolic extracts. Raw materials such as peach, stevia, citric acid, carboxymethyl cellulose (CMC), sodium benzoate, food flavor, and color were used. Peaches were washed with running tap water, peeled, sliced, and blended with some water. The mixture was then heated for 5 minutes, followed by the sequential addition of citric acid, sodium benzoate, and CMC. Next, fruit chunks, stevia, food flavor, and color were added to enhance the taste and appearance of the drink. Finally, prepared chamomile and hibiscus extracts were mixed into the beverage.

**In-Vivo study:** The study involved 60 Sprague-Dawley rats (250-300g), which were divided into six groups (n=10). Diabetes was induced by injecting STZ (40 mg/kg) intraperitoneally. To prevent deterioration, STZ was injected right away within a few minutes after being dissolved in ice-cold citrate buffer (0.01 M citric acid, pH 4.5). In order to prevent early hypoglycemia, diabetic rats were given a glucose solution. The positive and negative control groups were simply provided with filtered water and a normal diet. To verify that diabetes had been induced, the FBG was measured. Rats were classified as diabetic if their FBG level was more than 200 mg/dL (Alacabey *et al.*, 2023). All groups were treated for 60 days, with their biochemical profiles monitored periodically. Ethical approval was granted by the Institutional Biosafety and Bioethical Committee (Ethical Issue No. 1767). Table 1 provides an overview of the groups and their respective treatments.

**Physical parameters:** Feed intake of the rats was monitored throughout the trial by removing any spilled food from the total diet. The intake of the functional drink for each group was measured using graduated bottles. Additionally, body weight measurements were recorded for the experimental groups throughout the study.

**Biochemical assessment:** Blood samples were collected from each group at the start, middle, and end of the research trial. Rats were anesthetized with chloroform, and 3 ml of blood was taken from the tail vein into EDTA tubes for analysis. A glucometer (OnCall® Ez II; S. no. 303S0014E09) was used to measure blood glucose levels (Abduallah *et al.*, 2023). Serum insulin was determined using the Insulin ELISA® kit (Calbiotech Inc, USA). Lipid profile such as Triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and total cholesterol (TC), liver biomarkers such as Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), kidney biomarkers, including serum creatinine and urea levels, were assessed using colorimetric assays, and absorbance was determined using a spectrophotometer (PG Instruments, T80) (Sobhy *et al.*, 2015).

**Histopathology:** After decapitation, pancreatic tissues were separated and stored in a solution of 10% formalin. The samples were thinned into 5 µm sections with a microtome after being dehydrated and embedded in paraffin wax. Stained using eosin and hematoxylin stains, used a light microscope (MCX 100, Micros Austria) to look for changes in morphology, including pancreatic acinar cell degeneration and necrosis, as well as loss and a reduction of Langerhans islet cells followed by (Ali and Mustafa, 2023).

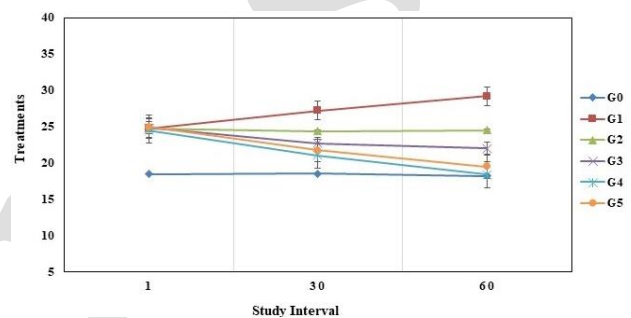
**Statistical analysis:** Using statistical software GraphPad Prism® 8.1, the obtained data for each parameter were subjected to two-way Analysis of variance (ANOVA) to determine whether the effects of treatments change over time, with treatments being factor one and study duration second. In addition, Tukey's HSD test was employed to determine the differences in the sample means as specified by Montgomery (2017).

## RESULTS

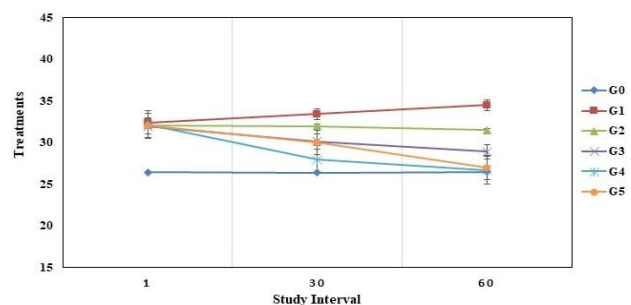
### Physical parameters

**Food and fluid intake:** Fig. 1(a) and (b) show the effect of treatments on the fluid and food intake of the experimental rats. In the positive control group (G<sub>1</sub>), both food and fluid intake significantly increased ( $P < 0.05$ ) over time. In contrast, the food and fluid intake in the chamomile-treated group (G<sub>2</sub>) showed no significant changes before and after treatment. By the end of the study, food and fluid intake decreased in the other diabetic-treated groups (G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub>).

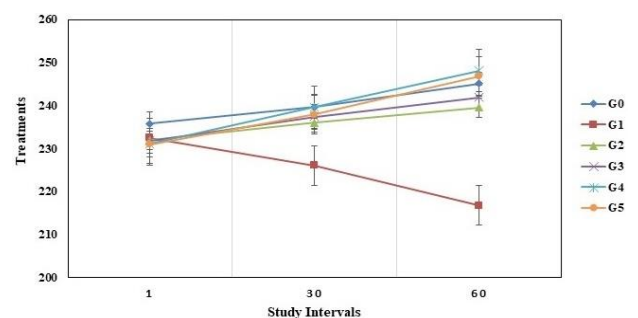
**Body weight:** Fig. 1(c) depicts the effects of ethanolic extract-based drinks of chamomile and hibiscus on body weight. All treatment groups (G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub>) of experimental rats showed a significant ( $P < 0.05$ ) increasing trend in body weight over the course of 60-day trial. The body weight of the diabetic control group (G<sub>1</sub>) decreased by the end of the trial.



**Fig. 1 (a):** Effects of chamomile and hibiscus on Food intake of diabetic rats (g). Treatment groups: G<sub>0</sub>= Negative control group, G<sub>1</sub>= Positive control group, G<sub>2</sub>= Positive group+ Peach drink containing Chamomile extract, G<sub>3</sub>= Positive group+ Peach drink containing Hibiscus extract, G<sub>4</sub>= Positive group+ Peach drink containing Chamomile and Hibiscus extracts, G<sub>5</sub>= Positive group+ standard drug (metformin).



**Fig. 1 (b):** Effects of chamomile and hibiscus on fluid intake of diabetic rats (mL).



**Fig 1 (c):** Effects of chamomile and hibiscus on body weight of diabetic rats (g).

**Random and fasting blood glucose level:** Table 2 shows the effects of chamomile and hibiscus extracts and their co-administration on the blood glucose level of diabetic rats. Data revealed a significant ( $P<0.05$ ) impact of treatments on random blood glucose (RBG) and fasting blood glucose (FBG) levels. Over the course of the trial, the RBG and FBG levels of the standard drug group ( $G_5$ ) and all value-added peach drink-given groups ( $G_2$ ,  $G_3$ ,  $G_4$ ) depicted a significant decrease in RBG and FBG levels. However, findings of the synergistic effect of the chamomile and hibiscus treated group ( $G_4$ ) were near to the RBG and FBG levels of rats given standard drug treatment (metformin) ( $G_5$ ).

**Serum insulin level:** Table 2 depicts the serum insulin levels of the experimental rats. A significant reduction in serum insulin was observed in the positive control group ( $G_1$ ) by the end of the trial. In contrast, serum insulin levels increased significantly ( $P<0.05$ ) in all treated groups ( $G_2$ ,  $G_3$ ,  $G_4$ , and  $G_5$ ). The combined administration of chamomile and hibiscus showed results comparable to those of metformin.

**Table 1:** Treatment plan for experimental rats

Groups	Treatments
$G_0$	Negative control
$G_1$	Positive control
$G_2$	Chamomile peach drink
$G_3$	Hibiscus peach drink
$G_4$	Chamomile and hibiscus peach drink
$G_5$	Metformin

**Table 2:** Means comparison for random and fasting blood glucose (mg/dL) and serum insulin level ( $\mu$ U/mL)

	Groups	Days		
		1	30	60
RBG	$G_0$	104.52 $\pm$ 3.37 <sup>i</sup>	103.98 $\pm$ 1.93 <sup>i</sup>	109.71 $\pm$ 0.90 <sup>i</sup>
	$G_1$	277.99 $\pm$ 2.02 <sup>b</sup>	282.91 $\pm$ 3.36 <sup>b</sup>	299.23 $\pm$ 3.07 <sup>a</sup>
	$G_2$	276.67 $\pm$ 3.06 <sup>b</sup>	253.56 $\pm$ 2.23 <sup>c</sup>	198.33 $\pm$ 7.63 <sup>f</sup>
	$G_3$	275.25 $\pm$ 2.47 <sup>b</sup>	227.30 $\pm$ 3.10 <sup>d</sup>	195.63 $\pm$ 1.76 <sup>f</sup>
	$G_4$	274.87 $\pm$ 1.77 <sup>b</sup>	216.52 $\pm$ 2.87 <sup>e</sup>	167.53 $\pm$ 5.11 <sup>g</sup>
	$G_5$	277.96 $\pm$ 2.26 <sup>b</sup>	197.66 $\pm$ 6.03 <sup>f</sup>	154.58 $\pm$ 1.86 <sup>h</sup>
FBG	$G_0$	91.91 $\pm$ 4.76 <sup>hi</sup>	92.29 $\pm$ 3.52 <sup>ghi</sup>	89.22 $\pm$ 0.63 <sup>i</sup>
	$G_1$	136.94 $\pm$ 1.29 <sup>b</sup>	144.66 $\pm$ 1.65 <sup>a</sup>	148.84 $\pm$ 1.4 <sup>a</sup>
	$G_2$	134.28 $\pm$ 1.34 <sup>bc</sup>	128.91 $\pm$ 1.79 <sup>cd</sup>	122.58 $\pm$ 2.17 <sup>de</sup>
	$G_3$	135.11 $\pm$ 1.91 <sup>bc</sup>	123.91 $\pm$ 1.19 <sup>de</sup>	111.56 $\pm$ 2.91 <sup>f</sup>
	$G_4$	135.52 $\pm$ 1.79 <sup>bc</sup>	122.04 $\pm$ 2.01 <sup>e</sup>	99.19 $\pm$ 1.30 <sup>gh</sup>
	$G_5$	134.74 $\pm$ 1.73 <sup>bc</sup>	119.84 $\pm$ 3.96 <sup>g</sup>	98.58 $\pm$ 1.18 <sup>g</sup>
Serum insulin	$G_0$	13.56 $\pm$ 0.45 <sup>a</sup>	13.6 $\pm$ 0.35 <sup>a</sup>	13.71 $\pm$ 0.04 <sup>a</sup>
	$G_1$	7.16 $\pm$ 0.44 <sup>gh</sup>	5.81 $\pm$ 0.12 <sup>hi</sup>	4.51 $\pm$ 0.22 <sup>i</sup>
	$G_2$	6.25 $\pm$ 0.24 <sup>h</sup>	9.81 $\pm$ 0.69 <sup>def</sup>	11.22 $\pm$ 0.49 <sup>bcd</sup>
	$G_3$	6.30 $\pm$ 0.61 <sup>h</sup>	8.31 $\pm$ 0.42 <sup>g</sup>	9.13 $\pm$ 0.45 <sup>ef</sup>
	$G_4$	6.34 $\pm$ 0.60 <sup>h</sup>	10.30 $\pm$ 0.33 <sup>cde</sup>	11.82 $\pm$ 0.50 <sup>bc</sup>
	$G_5$	6.29 $\pm$ 0.61 <sup>h</sup>	11.18 $\pm$ 0.59 <sup>cd</sup>	11.94 $\pm$ 0.38 <sup>ab</sup>

Means with similar letters in a row or column are statistically non-significant ( $P>0.05$ ). Treatment groups:  $G_0$ =Negative control group,  $G_1$ =Positive control group,  $G_2$ = Positive group+ Peach drink containing Chamomile extract,  $G_3$ =Positive group+ Peach drink containing Hibiscus extract,  $G_4$ =Positive group+ Peach drink containing Chamomile and Hibiscus extracts,  $G_5$ = Positive group+ standard drug (metformin).

**Lipid profile:** Table 3 represents the serum lipid profile of experimental rats by measuring the serum LDL, HDL, total cholesterol (TC), and triglycerides (TG). All treatments had a significant ( $P<0.05$ ) impact on serum LDL, TC, and TG. Their levels increased in the positive control group  $G_1$  and remained highest throughout the research trial. Whereas, reduced in all treated groups  $G_2$ ,  $G_3$ ,  $G_4$ , and  $G_5$  with time. The outcomes of the combined administration of both chamomile and hibiscus ( $G_4$ ) showed the nearest results to drug metformin ( $G_5$ ). Table 3 also depicts the effects

of treatments on the serum HDL level of experimental rats. A significant decrease in serum HDL level was seen in the positive control group ( $G_1$ ) as compared to the negative control group ( $G_0$ ). The HDL level significantly ( $P<0.05$ ) increased after treatment with ethanolic extracts-based drinks of chamomile and hibiscus and their combination. The group ( $G_4$ ) that was treated with the combination of chamomile+hibiscus showed the nearest results to that of the standard drug, metformin.

**Liver biomarkers:** Table 4 demonstrates the effects of chamomile and hibiscus on liver biomarkers. The levels of AST, ALT, and ALP significantly increased in positive control rats. All liver biomarkers were significantly ( $P<0.05$ ) reduced after treatment in groups  $G_2$ ,  $G_3$ ,  $G_4$ , and  $G_5$ , by the end of the study.

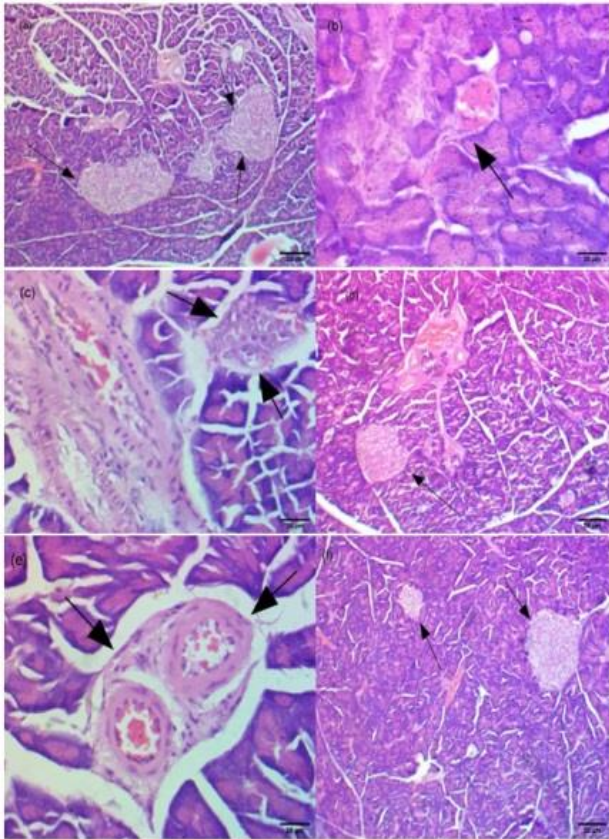
**Table 3:** Means comparison for Lipid profile (mg/dL)

	Groups	Days		
		1	30	60
LDL	$G_0$	29.49 $\pm$ 0.96 <sup>gh</sup>	29.98 $\pm$ 2.17 <sup>gh</sup>	29.2 $\pm$ 2.62 <sup>gh</sup>
	$G_1$	60.59 $\pm$ 2.72 <sup>c</sup>	72.83 $\pm$ 1.87 <sup>b</sup>	79.39 $\pm$ 1.24 <sup>a</sup>
	$G_2$	61.32 $\pm$ 1.62 <sup>c</sup>	46.82 $\pm$ 3.53 <sup>d</sup>	36.6 $\pm$ 0.63 <sup>ef</sup>
	$G_3$	60.55 $\pm$ 1.62 <sup>c</sup>	43.64 $\pm$ 0.88 <sup>d</sup>	34.32 $\pm$ 2.15 <sup>fg</sup>
	$G_4$	61.12 $\pm$ 1.49 <sup>c</sup>	40.92 $\pm$ 1.48 <sup>de</sup>	31.54 $\pm$ 0.88 <sup>gh</sup>
	$G_5$	62.15 $\pm$ 2.35 <sup>c</sup>	37.23 $\pm$ 2.59 <sup>ef</sup>	26.42 $\pm$ 1.60 <sup>h</sup>
HDL	$G_0$	44.95 $\pm$ 1.16 <sup>a</sup>	44.41 $\pm$ 1.22 <sup>a</sup>	44.18 $\pm$ 1.21 <sup>a</sup>
	$G_1$	28.14 $\pm$ 0.59 <sup>gh</sup>	25.84 $\pm$ 0.70 <sup>hi</sup>	22.80 $\pm$ 0.72 <sup>i</sup>
	$G_2$	27.64 $\pm$ 0.50 <sup>gh</sup>	29.36 $\pm$ 1.57 <sup>efg</sup>	32.48 $\pm$ 1.09 <sup>de</sup>
	$G_3$	28.14 $\pm$ 0.7 <sup>ef</sup>	31.51 $\pm$ 1.22 <sup>ef</sup>	36.05 $\pm$ 0.14 <sup>c</sup>
	$G_4$	28.3 $\pm$ 1.08 <sup>gh</sup>	34.91 $\pm$ 1.25 <sup>cd</sup>	40.78 $\pm$ 1.22 <sup>b</sup>
	$G_5$	28.69 $\pm$ 0.66 <sup>gh</sup>	35.65 $\pm$ 0.95 <sup>cd</sup>	43.94 $\pm$ 1.76 <sup>ab</sup>
TC	$G_0$	50.23 $\pm$ 4.55 <sup>ghi</sup>	50.44 $\pm$ 5.44 <sup>ghi</sup>	52.01 $\pm$ 5.53 <sup>ghi</sup>
	$G_1$	72.49 $\pm$ 1.93 <sup>bc</sup>	79.24 $\pm$ 3.48 <sup>ab</sup>	87.25 $\pm$ 4.42 <sup>a</sup>
	$G_2$	70.81 $\pm$ 2.09 <sup>bcd</sup>	67.47 $\pm$ 2.08 <sup>defg</sup>	60.32 $\pm$ 2.31 <sup>defg</sup>
	$G_3$	72.04 $\pm$ 3.22 <sup>bc</sup>	65.08 $\pm$ 3.32 <sup>cdef</sup>	52.28 $\pm$ 2.98 <sup>ghi</sup>
	$G_4$	71.36 $\pm$ 3.10 <sup>bc</sup>	58.59 $\pm$ 1.01 <sup>efgh</sup>	48.71 $\pm$ 1.31 <sup>hi</sup>
	$G_5$	70.79 $\pm$ 6.56 <sup>bcd</sup>	55.29 $\pm$ 1.21 <sup>fgh</sup>	44.19 $\pm$ 2.57 <sup>i</sup>
TG	$G_0$	53.66 $\pm$ 0.92 <sup>g</sup>	54.45 $\pm$ 2.31 <sup>g</sup>	52.61 $\pm$ 1.20 <sup>g</sup>
	$G_1$	77.19 $\pm$ 1.89 <sup>b</sup>	94.8 $\pm$ 2.96 <sup>a</sup>	100.79 $\pm$ 3.36 <sup>a</sup>
	$G_2$	75.45 $\pm$ 3.45 <sup>bc</sup>	69.22 $\pm$ 1.76 <sup>cd</sup>	63.01 $\pm$ 2.47 <sup>def</sup>
	$G_3$	76.39 $\pm$ 1.63 <sup>bc</sup>	66.81 $\pm$ 3.03 <sup>de</sup>	59.06 $\pm$ 3.10 <sup>efg</sup>
	$G_4$	75.39 $\pm$ 2.98 <sup>bc</sup>	65.30 $\pm$ 1.85 <sup>de</sup>	55.64 $\pm$ 2.64 <sup>g</sup>
	$G_5$	74.86 $\pm$ 1.35 <sup>bc</sup>	62.85 $\pm$ 2.42 <sup>def</sup>	53.34 $\pm$ 3.70 <sup>fg</sup>

**Kidney biomarkers:** Table 5 presents the results related to kidney biomarkers (Urea, Creatinine). Diabetes significantly increased serum urea and creatinine levels compared to the negative control group ( $G_0$ ). Both kidney biomarkers increased in the diabetic control group ( $G_1$ ) by the end of the research trial (60th day). In contrast, both were significantly decreased in diabetic-treated groups  $G_2$ ,  $G_3$ ,  $G_4$ , and  $G_5$  following treatment with chamomile and hibiscus. Furthermore, mean comparisons of urea indicate no significant difference between the group receiving combined chamomile and hibiscus ( $G_4$ ), and the standard drug metformin ( $G_5$ ), indicating that both treatments are equally effective in reducing serum urea levels.

**Histopathology:** Fig. 2 (a-f) illustrates the histopathological findings of rat pancreatic tissues across all treatment groups. Fig. 2 (a) shows the pancreas of the control group ( $G_0$ ) indicating the presence of entirely active islets in the pancreatic parenchyma, as well as intact pancreatic acinar cell structure. Fig. 2 (b)





**Fig. 2 (a-f):** Histopathological examination of rat's pancreatic tissues at 40x. While pointed structures represent insulin secreting units and Scale bar= 20µm. (a) Histopathology of negative control rats (b) Histopathology of positive control rats (c) Histopathology of Chamomile treated rats (d) Histopathology of Hibiscus treated rats (e) Histopathology of combination of both Chamomile and Hibiscus treated rats (f) Histopathology of drug-treated (metformin) rats.

**Table 4: Means comparison for Liver biomarkers (mg/dL)**

	Groups	Days		
		1	30	60
AST	G <sub>0</sub>	29.69±1.03 <sup>h</sup>	31.72±3.82 <sup>h</sup>	32.51±2.57 <sup>h</sup>
	G <sub>1</sub>	65.01±4.33 <sup>bc</sup>	70.89±1.49 <sup>b</sup>	82.76±2.62 <sup>a</sup>
	G <sub>2</sub>	60.89±2.5 <sup>cde</sup>	49.23±1.15 <sup>f</sup>	35.14±3.53 <sup>h</sup>
	G <sub>3</sub>	62.28±3.28 <sup>bcd</sup>	53.69±3.55 <sup>def</sup>	48.09±4.25 <sup>f</sup>
	G <sub>4</sub>	60.13±1.53 <sup>cde</sup>	52.22±2.14 <sup>ef</sup>	37.71±3.1 <sup>gh</sup>
	G <sub>5</sub>	61.06±4.00 <sup>cd</sup>	44.99±1.25 <sup>fg</sup>	31.72±1.71 <sup>h</sup>
ALT	G <sub>0</sub>	35.31±0.79 <sup>i</sup>	34.26±1.19 <sup>i</sup>	34.96±3.94 <sup>i</sup>
	G <sub>1</sub>	59.76±1.14 <sup>abcd</sup>	62.18±1.92 <sup>ab</sup>	65.76±1.68 <sup>a</sup>
	G <sub>2</sub>	58.92±1.63 <sup>abcd</sup>	53.07±1.22 <sup>cdef</sup>	46.03±3.55 <sup>gh</sup>
	G <sub>3</sub>	58.61±3.11 <sup>abcd</sup>	55.84±2.20 <sup>bcd</sup>	52.56±4.27 <sup>def</sup>
	G <sub>4</sub>	60.90±2.10 <sup>abc</sup>	52.82±3.63 <sup>def</sup>	41.69±2.72 <sup>ghi</sup>
	G <sub>5</sub>	59.57±2.55 <sup>abcd</sup>	48.73±1.93 <sup>efg</sup>	38.55±2.98 <sup>hi</sup>
ALP	G <sub>0</sub>	134.17±2.13 <sup>h</sup>	133.52±3.67 <sup>h</sup>	133.63±1.56 <sup>h</sup>
	G <sub>1</sub>	171.65±1.16 <sup>bc</sup>	174.45±0.9 <sup>ab</sup>	180.44±1.22 <sup>a</sup>
	G <sub>2</sub>	173.60±0.89 <sup>b</sup>	165.45±2.22 <sup>c</sup>	157.47±2.4 <sup>d</sup>
	G <sub>3</sub>	174.53±2.72 <sup>ab</sup>	152.92±2.91 <sup>de</sup>	143.71±3.9 <sup>fg</sup>
	G <sub>4</sub>	172.16±1.77 <sup>bc</sup>	149.03±1.83 <sup>ef</sup>	139.04±1.33 <sup>gh</sup>
	G <sub>5</sub>	171.98±1.74 <sup>bc</sup>	145.29±2.47 <sup>fg</sup>	133.29±1.91 <sup>h</sup>

demonstrates that rats in the positive control group (G<sub>1</sub>) exhibited significant architectural disturbances and shrinkage of islet cells. Fig. 2 (c) shows the pancreatic tissues of rats in chamomile-treated group (G<sub>2</sub>), where there is a resolution of infiltrated immune mass with slight cellular hyperplasia. Mild reductions in fatty changes and inflammatory cell infiltration are also observed. Fig. 2 (d) depicts the pancreas of rats in the Hibiscus-treated group (G<sub>3</sub>) which shows immune mass

infiltration and recovery of adjacent luminal surfaces with cellular hyperplasia. Fig. 2 (e) presents the pancreas of rats treated with a combination of chamomile and hibiscus extracts (G<sub>4</sub>), showing moderate normalization of pancreatic tissues. There is recovery with hyperplasia, disrupted cellular integrity of white cells, and, clearance of immune bodies. Fig. (f) illustrates the histopathological changes in the pancreatic parenchyma of drug-treated rats (G<sub>5</sub>). Compared to the positive control group, less damage is observed in the pancreatic tissues, with a marked reduction in fatty changes and inflammatory cell infiltration.

**Table 5: Means comparison for Kidney biomarkers (mg/dL)**

	Groups	Days		
		1	30	60
Urea	G <sub>0</sub>	18.69±1.00 <sup>g</sup>	17.40±1.01 <sup>g</sup>	18.40±0.95 <sup>g</sup>
	G <sub>1</sub>	33.78±1.19 <sup>abc</sup>	36.80±0.63 <sup>ab</sup>	38.39±0.82 <sup>a</sup>
	G <sub>2</sub>	33.07±1.61 <sup>abc</sup>	28.91±3.07 <sup>cde</sup>	25.42±4.60 <sup>def</sup>
	G <sub>3</sub>	31.24±1.35 <sup>bcd</sup>	27.31±2.00 <sup>cdef</sup>	21.51±4.06 <sup>g</sup>
	G <sub>4</sub>	32.4±1.12 <sup>abc</sup>	23.69±0.99 <sup>efg</sup>	20.9±2.12 <sup>g</sup>
	G <sub>5</sub>	32.54±0.57 <sup>abc</sup>	22.86±2.89 <sup>efg</sup>	18.45±0.87 <sup>g</sup>
Creatinine	G <sub>0</sub>	0.98±0.09 <sup>efg</sup>	0.85±0.06 <sup>g</sup>	0.88±0.12 <sup>g</sup>
	G <sub>1</sub>	1.46±0.04 <sup>b</sup>	1.82±0.16 <sup>a</sup>	2.05±0.11 <sup>a</sup>
	G <sub>2</sub>	1.45±0.06 <sup>b</sup>	1.34±0.04 <sup>bcd</sup>	1.21±0.12 <sup>bcd</sup>
	G <sub>3</sub>	1.44±0.02 <sup>bc</sup>	1.26±0.05 <sup>bcd</sup>	1.18±0.08 <sup>cdef</sup>
	G <sub>4</sub>	1.45±0.02 <sup>bcd</sup>	1.21±0.05 <sup>bcd</sup>	0.95±0.08 <sup>fg</sup>
	G <sub>5</sub>	1.43±0.04 <sup>b</sup>	1.18±0.04 <sup>def</sup>	0.89±0.01 <sup>g</sup>

## DISCUSSION

Biochemical screening can provide the information required for the diagnosis of disease and effects of the treatments.

Body weight loss is one of the most common symptoms of diabetes, resulting from the body's inability to properly metabolize glucose. As glucose cannot be effectively utilized, the body begins to break down fat and muscle for energy, leading to a decrease in body weight (Abduallah *et al.*, 2023). Studies have shown that treatment with chamomile and hibiscus can significantly increase body weight, and this weight gain may be attributed to the protective effects of chamomile extract, which could involve the prevention of muscle wasting. Potential mechanisms include the reversal of gluconeogenesis and glycogenolysis, along with improvements in insulin secretion and glycogen control. Additionally, the lack of insulin leads to various metabolic abnormalities in animals, including elevated blood glucose, decreased protein content, and increased triglyceride and cholesterol levels (Tenpe and Yeole, 2009). In another study by Sachdewa and Khemani (2003), rats treated with Hibiscus showed a significant increase in body weight.

The food and fluid intake of the hibiscus treated diabetic rats returned to normal, comparable to the values of the non-diabetic group (Afiune *et al.*, 2017). In contrast, chamomile given to diabetic rats showed no significant change in either parameter before and after treatment (Hassan Al-Musa and Fahaid Al-Hashem, 2014).

Hyperglycemia is a primary sign of diabetes and a major contributor to diabetes-related complications; therefore, managing it is important for effective disease control (Abduallah *et al.*, 2023). Chamomile and hibiscus

have been shown to improve hyperglycemia in various studies, such as during the biochemical analysis of laying Japanese quails, chamomile extract showed a reduction in serum glucose level (Ezeldien *et al.*, 2023). Based on studies of both experimental and clinical reports, Chamomile extracts and essential oils were found to have antidiabetic effects (Ivanović *et al.*, 2014). Furthermore, in another study, chamomile showed antidiabetic effects (Ahmad *et al.*, 2020). Chamomile tea had a significant impact on glycemic control in type 2 diabetic patients (Zemestani, 2015). In another study, chamomile tea showed a significant decrease in blood sugar levels in alloxan-induced hyperglycemic rats (Khan, 2014). Moreover, oral administration of chamomile oil significantly reduced the blood sugar level and increased serum insulin level in STZ-induced diabetic rabbits (Saghahazrati *et al.*, 2020). Another trial proved that hibiscus has potential as an alternative treatment for diabetes (Chauhan and Rani, 2024).

The fundamental mechanism of chamomile is associated with the reduction in blood glucose levels by stimulating peripheral glucose utilization, particularly in muscle and adipose tissue (Ramadan and Eman, 2012). Alternatively, it may also involve the restoration of enzyme function that aids in the metabolism of glucose and glycogen. Essentially, the antioxidants seem to have a role in reducing the effect of oxidative damage and blood glucose levels by affecting peripheral glucose use and aiding in the restoration of enzyme activity in glucose-related tissues. This phenomenon occurs by the enzymatic activation of sorbitol dehydrogenase and aldose reductase (Yan *et al.*, 2017). Moreover, chamomile extract is composed of flavonoids i.e. apigenin, apigenin-7-o-glucoside, cis and trans-2-hydroxy-4-methoxycinnamic glucoside which inhibit the activity of enzymes ( $\alpha$ -amylase and maltase) (El Mihaoui *et al.*, 2022). The highest inhibition of the aforementioned enzymes was observed from apigenin and apigenin-7-o-glucoside respectively. Additionally, both flavonoids were able to control sugar absorption and limit sucrose and glucose transport. In a separate study, it was found that the hydro-methanolic extract of chamomile as well as certain isolated components, demonstrated the ability to inhibit the activity of the enzyme aldose reductase in rats (Hwang *et al.*, 2018).

The fundamental mechanism of hibiscus may be due to the presence of polyphenols that might lower blood glucose levels. The phenolic chemicals included in hibiscus are  $\alpha$ -amylase and  $\alpha$ -glucosidase, these two major enzymes involved in carbohydrate digestion. Polyphenols also prevent intestinal Caco-2 cells from absorbing glucose. Tannic acid, which promotes the translocation of glucose transporters (GLUT 4) and activates insulin receptors via phosphorylation, is primarily responsible for circulating glucose clearance. As a result, the polyphenols and antioxidants found in hibiscus are primarily responsible for the plant's ability to manage blood sugar levels. Another study also suggests that hibiscus extract might inhibit  $\alpha$ -amylase activity, potentially contributing to its anti-diabetic benefits (Harini, 2024). Furthermore, the hibiscus plant as a whole improves the pancreatic beta cells' ability to secrete

insulin, most likely due to the effects of polyphenolic chemicals, terpenoids, flavonoids, and alkaloids (Solangaarachchi, 2022).

The lipid profile is an important factor in diabetes, as elevated levels of cholesterol, and LDLs are commonly observed in diabetic patients. Monitoring these levels is essential for evaluating the complications associated with diabetes (Abduallah *et al.*, 2023). Similar outcomes related to chamomile effect on lipid biomarkers have been reported in studies by Mukesh and Payil (2011), Mamun *et al.* (2013), Elsemelawy (2017), and Prasanna *et al.* (2017), while hibiscus has been investigated in studies by Sankaran (2011) and Zaki *et al.* (2017). The aqueous extract of chamomile contains  $\beta$ -glucan, a water-soluble fiber known for its beneficial effects. The reduction of cholesterol levels after treatment with chamomile extract may be due to  $\beta$ -glucan that creates a viscous layer in the small intestine (Rondanelli *et al.*, 2009). This layer reduces the uptake of dietary cholesterol while promoting increased production of bile acid and lowering blood cholesterol. According to EFSA (2010), consumption of oat-derived  $\beta$ -glucan can lower blood cholesterol and reduce the risk of heart coronary diseases. Moreover, Afiune *et al.* (2017) suggest that Tannin and anthocyanin serve as the main antioxidant constituents within the extract of hibiscus flowers. The elevation of HDL levels might be linked to the effects of anthocyanin, as studies involving anthocyanin supplementation in both humans and animals have shown similar outcomes, demonstrating enhancements in atherogenic activity attributed to their influence on reverse cholesterol transport.

Liver and kidney biomarkers were also evaluated to check the protective effect of both herbs. Chamomile extracts can effectively lower blood sugar levels, enhance insulin production, and regulate liver enzymes in the blood. It may positively contribute to have a positive effect on the management of diabetes without causing harm to the liver (Annabestani *et al.*, 2023). The decrease in serum creatinine and urea levels in diabetic rats treated with chamomile could be due to its antioxidant properties (Najla *et al.*, 2012). Hibiscus extract also may restore kidney damage in STZ-induced diabetic rats. Its extract demonstrated significant anti-diabetic properties by lowering blood glucose and other toxic metabolic waste, such as uric acid, creatinine, urea, and alanine transaminase, which are byproducts of the body's compensatory response to hyperglycemia (Viado *et al.*, 2022). Similarly, Zaki *et al.* (2017) also proved the kidney protective effects of hibiscus in diabetic rats.

**Conclusions:** Chamomile and hibiscus exhibit promising antidiabetic effects, with the regulation of glucose and lipid metabolism, suggesting their potential as complementary therapies for diabetes management. Furthermore, with the growing interest in herbal alternatives to conventional medications, combining both herbs synergistically may provide a safer and more convenient option for people managing diabetes, particularly in situations where pharmaceuticals may not be as readily available or for patients seeking natural alternatives.

**Credit author statement:** Zaema Yasin: Experimentation; Data management; Statistical analysis; Conceptualization; Writing-original draft. Moazzam Rafiq Khan: Conceptualization; Funding acquisition; Validation; Supervision; Visualization; Project administration, Writing-review. Muhammad Asim Shabbir: Conceptualization; Writing-review; Supervision; Resources. Beenish Israr: Conceptualization; Writing - review & editing.

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