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BIOCHEMICAL INVESTIGATION OF ANTI DIABETIC EFFECTS OF GINKGOLIDE B IN EXPERIMENTALDIABETIC ANIMALS

Asma Yousaf[1], Muhammad Tariq[1] Faiza Mumtaz[2,3], Usman Javaid[4,5], Hassan Ahmed[6], Sakeena Noor[7], Farzana Khan[6], Maddiha Nasir[8], Muhammad Ibrahim[9], Hafiz Muhammad Usman Abid[10]

[1]*Faculty of Pharmaceutical Sciences, Times Institute, Multan*
[2]*Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Lyallpur College of Pharmaceutical Sciences, Faisalabad.*
[4]*Faculty of Pharmacy and Pharmaceutical sciences, Department of Pharmacology, University of Karachi*
[5]*KAIMS International Institute, Multan*
[6]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University, Multan*
[7]*College of Pharmacy, University of Sargodha*
[8]*Department of Pharmacology, Faculty of Pharmacy, Bahauddin Zakariya University, Multan*
[9]*Riphah Institute of Pharmaceutical Sciences, Riphah University, Lahore*
[10]*Health Services Academy, Islamabad, Pakistan*
[11]*Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan*

Corresponding author:
Hafiz Muhammad Usman Abid (usman.abid@hsa.edu.pk)

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Abstract

Diabetes mellitus is a chronic condition characterized by persistent hyperglycemia due to insufficient insulin production, secretion, or resistance. Despite advances in treatment, the global prevalence of diabetes continues to rise, underscoring the need for novel therapeutic strategies. Ginkgolide B, a bioactive compound derived from Ginkgo biloba, has demonstrated significant anti-inflammatory and antioxidant properties, which are closely linked to diabetes management. This study evaluated the pharmacological effects of Ginkgolide B as an antidiabetic, antioxidant, and anti-inflammatory agent in an alloxan-induced diabetic rat model. Male Wistar rats were divided into five groups: normal control, diabetic control, Glibenclamide-treated group (5 mg/kg), and two Ginkgolide B-treated groups (5 mg/kg and 10 mg/kg). Diabetes was induced by intraperitoneal administration of alloxan monohydrate (120 mg/kg). Key parameters, including fasting blood glucose (FBG), lipid profile, antioxidant activity, and paw edema, were assessed alongside histopathological evaluations. Treatment with Ginkgolide B significantly lowered FBG levels in diabetic rats, with the higher dose (10 mg/kg) reducing FBG from a baseline average of 311 mg/dL to 155 mg/dL by day 21. Lipid profile analysis revealed an increase in triglycerides and HDL levels in Ginkgolide B-treated groups, particularly at the higher dose. Ginkgolide B also exhibited potent antioxidant activity, achieving 70% radical scavenging at a concentration of 50 µg/mL, comparable to ascorbic acid. Additionally, Ginkgolide B reduced paw edema in the anti-inflammatory assessment, with effects comparable to those of indomethacin at higher dosages. These findings indicate that Ginkgolide B possesses antidiabetic, antioxidant, anti-inflammatory, and lipid-modulatory effects in diabetic rats. This suggests its potential as a therapeutic agent for diabetes management. Further studies are warranted to elucidate its mechanisms of action and assess its efficacy in clinical settings.

Keywords:
Ginkgolide B, diabetes, antioxidant, anti-inflammatory, lipid profile, oxidative stress.

INTRODUCTION

Diabetes mellitus (DM) is a major global health problem that has not yet reached its peak, affecting around 800 million people worldwide. The condition is primarily characterized by abnormal blood sugar levels brought on either due to a lack of insulin or an inability of the body to properly use insulin[1]. Type 1 diabetes is due to destruction of β-cell in the pancreas, where it is unable to secret insulin and type 2 diabetes is associated with Insulin Resistance and β-cell dysfunction. There are 463 million adults with diabetes around the world in 2019 and the figure is projected to increase to 700 million by 2045 [2]. Sedentary lifestyle, unhealthy eating habits and rising obesity is some of the reasons behind this increase. Many of the complications of diabetes, such as retinopathy, nephropathy, cardiovascular disease, and neuropathy, contribute to the high morbidity and mortality rates associated with it [3]. The financial price of diabetes is hefty as well. The IDF stated that global diabetes expense topped ~$760 billion in 2019, and will continue to climb with more and more individuals suffering from the disease [3, 4]. The costs may be direct-hospitalizations, prescription drugs, medical treatments, or it can be an indirect cost, including loss of productivity due to illness, disability and premature death. But this financial burden is putting healthcare systems under considerable pressure, particularly in low- and middle-income countries, where diabetes is increasing at a rapidly accelerating pace [5, 6]. At present, diabetes management is predominantly focused on insulin therapy, oral hypoglycemic drugs and lifestyle interventions including diet and physical activity. Type 1 diabetes is still mainly treated with insulin replacement therapy while Type 2 is managed by lifestyle changes such as exercise and weight loss. As the disease progresses, insulin therapy or oral antidiabetic medications are often required. Common oral medications include metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists. However, there are numerous challenges to these therapies. As type 2 diabetes is a progressive condition and glycaemic control may worsen with time, more intensive treatment regimens are needed and may entail insulin or combination therapy. Moreover, control of blood sugar levels is still inadequate for many patients and diabetes-related complications continue to affect the quality of life of many, highlighting that existing therapies fail to resolve the etiology of the disease [7]. Oxidative stress and inflammation in both the onset and progression of diabetes have been highlighted by recent studies. Chronic hyperglycemia causes over production of reactive oxygen species (ROS), which are subsequent to oxidative stress and damage to proteins, lipids and DNA [8]. This oxidative stress decreases insulin sensitivity and β-cell function and induces complications including cardiovascular disease and retinopathy. Further contributing to insulin resistance and -cell dysfunction, chronic low-grade inflammation is often linked to obesity and Type 2 diabetes[8]. These complications call for novel therapeutic approaches targeting mechanisms implicated in the pathophysiology of diabetes, including inflammation, oxidative stress and β-cell dysfunction. Such methods could improve glycaemic control, patient compliance, and reduce side effects. One interesting area of research can be the development of therapies to modulate inflammation and oxidative stress. Antioxidant therapies are being evaluated as a means of enhancing the body to combat its own naturally occurring antioxidant defenses while also reducing the level of ROS produced. These anti-inflammatory treatments are aimed at suppressing the chronic inflammation associated with insulin resistance and β-cell dysfunction. Substances such as Ginkgolide B, Resveratrol and Curcumin are being studied for their ability to modulate these pathways. Personalised medicine has become increasingly important in the context of Type 2 diabetes, for which treatment response is highly variable[9]. Personalised medicine aims to tailor treatments based on an individual patient's distinct profile in order to optimise efficacy and minimise adverse effects. Ginkgo biloba (GB) includes a bioactive component, ginkgolide B, and possesses high levels of anti-inflammatory and antioxidant activities and GB may provide beneficial effects on diabetes[10]. Ginkgolide B ablating ROS and reducing redox stress is found to be one of the major shooter of diabetes and its associated complications. It preserves β-cell function, which is needed for insulin secretion, and protects against oxidative damage. In addition, Ginkgolide B acts as an anti-inflammatory factor which may be able to regulate the chronic inflammation of the overweight environment that is hypothesized to underlie β-cell dysfunction and insulin resistance in diabetes[11]. While further research is needed to verify these impacts, initial studies suggest Ginkgolide B can even help boost insulin sensitivity and blood glucose management. In addition to its anti-inflammatory and antioxidant effects, ginkgolide B may help prevent or delay diabetic complications such as retinopathy, neuropathy, and nephropathy. By reducing inflammation and oxidative stress, ginkgolide B might protect against injury to the kidneys, nerves and retinal cells. Focusing on its therapeutic potential, which remains largely unexplored, we will perform lit review to clarify the effects of MT on the sc and its clinical relevance; and conclude that this is a potential new target f delivering an effective treatment. One limitation that may hinder

its applicability is its low oral bioavailability[12]. Further exploring potential delivery strategies, such as open in a new window nanoparticles or controlled-release formulations, may optimize delivery properties to improve therapeutic efficacy and bioavailability of the drug. However, the global epidemic of diabetes and the large health and economic morbidity burden it places on societies all indicate the urgent need for a new therapeutic approach. The main limitations of current treatments are their side effects and their inability to cure the underlying causes of the disease. Research into anti-inflammatory drugs, antioxidants, and precision medicine provides an optimistic outlook for improvements in diabetes care. Due to its anti-inflammatory and antioxidant properties, ginkgolide B has the potential to be used as novel treatment; however, further clinical studies are necessary to confirm the safety and efficacy of ginkgolide B for the treatment of diabetes and its complications.

Methods

Materials:

Ginkgolide B, Alloxan, Normal saline, syringes, Deionized water, Glucometer, Test kits, Placebo substances for control groups, Test strips, Glibenclamide

FTIR

It should be noted that FTIR or Fourier Transform Infrared Spectroscopy is an important analytical technique. is used to study the molecular vibrational frequency of a sample. It starts with preparing the sample in the right manner with the main focus on its appropriate form then it exposes it to infrared radiation. The transmitted or reflected radiation is than detected and converted into interferogram, which is the graphical representation of intensity with time. The following results in an interferogram image of the sample, and the data is further transformed into a spectrum that is finely studied to explain the molecular vibrational modes. Consequently, the typical FTIR spectrum is recorded within the 400–4000 cm–1 region with a resolution of 4 cm-1 and by co-accumulating 32 interferograms to improve the signal-to-noise ratio. Before analysis, the instrument is standardized with the help of mercury lamp and polyethylene film. The FTIR analysis is performed on the PerkinElmer Spectrum RXI spectrometer using diamond ATR accessory that enables identification of the characteristic absorption bands belonging to functional groups present within the sample.

In-vitro antioxidant activity of Ginkgolide B:

DPPH radical scavenging activity:

In order to measure the antioxidant potential of natural products, extracts and compounds, the method for determination of DPPH radical scavenging activity is one of the most widely applied techniques. To do this, dissolve 3. DPPH at the concentration of 1 mM was prepared by dissolving 84 mg of DPPH powder in 100 mL of methanol. The test material which could be an extract or a compound is further diluted serially in methanol to the required concentrations. Also, methanol is used to prepare a standard reference solution of Trolox or Vitamin C. Determination of the DPPH radical scavenging capacity of the samples or standards involves adding 200µl DPPH solution to 200 µl sample/standard solution in a microplate. The activity of the DPPH with the test material is initiated after incubation of the reaction mixture at room temperature (25 ° C) for thirty minutes. Thereafter, absorption of the resulting mixture is measured at 517 nm which infers the use of a spectrophotometer [13]. They in turn facilitates calculation of the scavenging percentage.

% radical scavenging activity=(Ac-As)/Acx100

On the other hand, AC and AS stand for the sample's and the control's respective absorbance.

Experimental animals:

Male albino for the study, wistar rats, in the age range of 9 – 10 weeks with average mean weight of 250 – 300 g were used. To acclimatise the animals, the animals were exposed to a controlled environment of 23± 2°C and a light-dark cycle of 12 hours as they stayed in the departmental animal facility before and during the experiments. They were provided unfettered access to water, and mean fed by pellets from monitored limited portions at defined intervals. In the conduct of the in vivo study, the protocols of the Institutional Animal Care Committee were adhered to as well as the protocols in the Guide for the Care and Use of Laboratory Animals.

Diabetes induction:

Diabetic rats were prepared by an intraperitoneal injection of 120 mg/kg of sterile normal saline (0. 9% NaCl) containing alloxan monohydrate for overnight fasted rats except the control group. To confirm the diagnosis of diabetes one week later fasting blood glucose was estimated using the glucose strip and an electronic glucometer (Accu-Check, Roche Diabetes Care GmbH, Germany). To augment the study, rats with fasting plasma glucose level of between 250 and 300 mg/dl were selected[14].

Experimental design and treatment schedule

The rats were split into the following compositions after being sorted into five groups (n = 6): Group-1, the standard control group of rat the received normal saline (5 ml/kg/day). The rats in Group 2 were alloxan induced diabetic and received regular saline. Rats in the diabetic Group 3 received glibenclamide at a concentration of 5mg/Kg. Rats in Group 4 which was the diabetic rats were treated with Ginkgolide B at a dose of 5 mg/ kg.

Group 5, consisting diabetic rats that received normal sugar diet were administered with Ginkgolide B (10 mg/kg). To the relevant groups, glibenclamide and ginkgolide B were given orally for 28 days, at a dosage of once per day.

Blood sampling and Biochemical analysis

In the entire study, blood samples of the rat tail vein were taken at some points in time. The general body weight and blood glucose level tests were conducted on the first, seventh, fourteenth 21st and twenty eighth day of the study. An electronic glucometer was used in the assessment of the blood glucose concentrations. Further, some biochemical profiles were estimated using fully automated analyser (ERBA-EM 200) with standard reagents and kits obtained from Erba Diagnostics. These parameters were total proteins, creatinine, urea, uric acid, total cholesterols, low density lipoprotein cholesterols, high density lipoprotein cholesterols and triglycerides.

Anti-inflammatory study

The Carrageenan-induced paw edema method is employed in rat to assess the in vivo anti-inflammatory effect. Rats are divided into different groups; the treatment group and the control group and the extent of the reduction in paw edema is then used to determine the anti-inflammatory effect. The rats in the treatment group receive single dose of the Ginkgolide B either orally, 1 hr before the induction of inflammation. Inflamed rat paw is assessed by injecting carrageenan subcutaneously into the paw pad of the rat and measuring paw volume after certain hours, most preferably 1 hour, 2 hours, 4 hours and 6 hours using a plethysimeter. The percentage inhibition of edema is determined from change in paw volume of the treatment group relative to the control group. The degree of inflammation is also determined with help of the measurement of the concentration of pro-inflammatory cytokines with ELISA in the serum. The results are statistically tested using t-test in order to compare if the findings are statistically significant and presented in mean ±SD.

Statistical analysis

To obtain reliability, each experiment has been repeated thrice in order to minimize any variation. Further statistical analysis to compare the differences was done with the help of Student's t-test. The findings are presented in form of mean ± SD. Also, with GraphPad Prism software with developed and installed in the computer version: 5. 01 (GraphPad Software, San Diego, CA, USA), software was used in the visualization of data and producing accurate and helpful graphs.

Results

FTIR

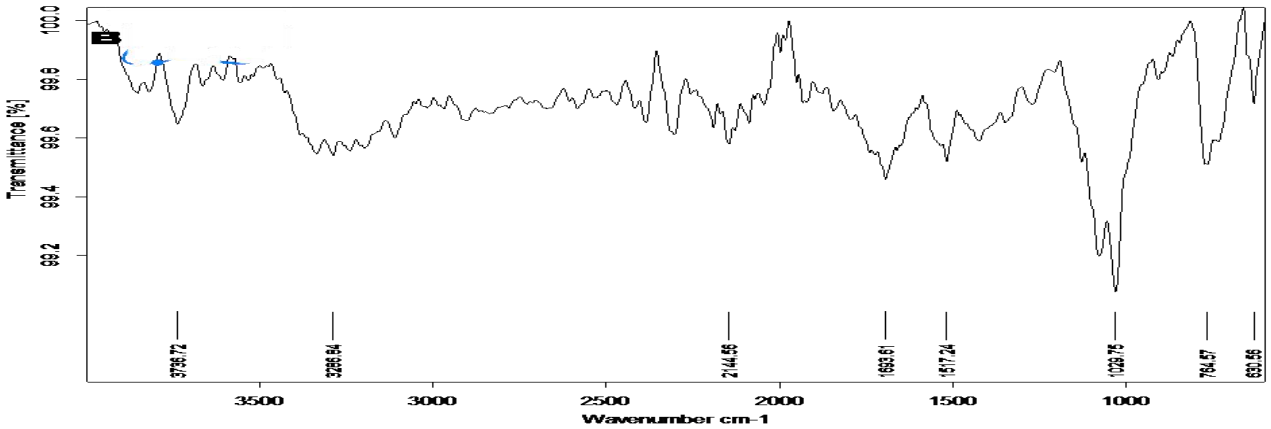


Figure 1:FTIR of ginkgolide B which shows the peaks of typical functional groups present in ginkgolide B

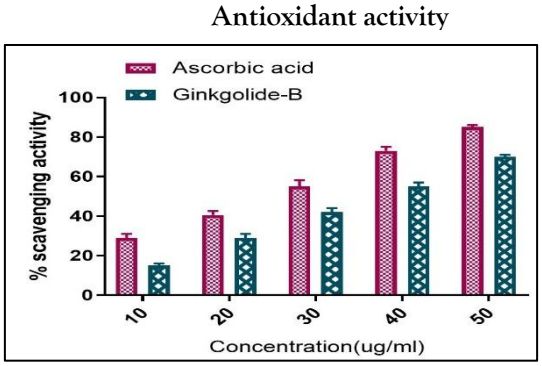
The observation of bond stretching and bending vibrations which reveals the functional groups present in any compound is facilitated by FTIR analysis. The analysed-spectrum of Ginkgolide B using FTIR exhibits different unique peaks as indicated below.

The carbonyl and hydroxyl stretching vibrations were identified where O-H stretching vibrations indicate the presence of hydroxyl groups (-OH) shown by a broad peak at about 3286 cm^{-1}. These groups are present in alcohols often, and as for ginkgolide B, it is a structure consisting of a terpene lactone, therefore, it likely has these as well. The values of 1693 cm^{-1} suggest that carbonyl (C=O) are stretching and it is commonly related to aldehydes, lactones or esters. The two lactone rings of ginkgolide B may assists in this absorption as well as other lactone compounds.

This is further proven by the peaks seen at 1029 cm^{-1} which correspond to C=O of ester and ether functional groups after diagramming the Ginkgolide B. Additionally, bands at 3438 cm^{-1} smaller peak at 3736 cm^{-1} are due to the C-H stretching in methyl in the structure an alkanes functional group.

In the fingerprint region between 1400 – 1000 cm^{-1} multiple complex bands can be observed representing

some of the ring, C–O and C–H bending vibrations. Such intricate pattern contributes to the affirmation of the exact bicyclic structure of Ginkgolide B.



The antioxidant potential of Ginkgolide B and ascorbic acid (standard compound used in the experiment) was assessed spectrophotometrically by the determination of free radical scavenging effects on DPPH. The findings were noteworthy. To further check the antioxidant activity of the compounds under study at varied concentration levels, experiments were carried out at 10 µg/ml, 20 µg/ml, 30 µg/ml and 50 µg/ml. A clear trend was observed that pointed out that an increase in the concentration of ascorbic acid and Ginkgolide B increases the antioxidant activity. The free radical scavenging activities of Ginkgolide B was found to be 15±1%, 29±2%, 42±2% and 55±1% at 10 µg/ml, 20 µg/ml, 30 µg/ml and 40µg/ml. Thus Ginkgolide B achieved 70±1% DPPH free radical scavenging activity at concentration of 50 µg/ml (Figure 2). This was the highest activity of any compound.

Figure 2:Antioxidant activity of Ginkgolide B and standard ascorbic acid. According to these results Ginkgolide B shows efficient antioxidant activity

Table 1: Fasting blood glucose levels of diabetic rats on Day 0,7,14 and 21.:

Groups	Day 0 (mg/dl)	Day 7 (mg/dl)	Day 14 (mg/dl)	Day 21 (mg/dl)
Normal control	107±2.5	108±3	111±2.8	114±2.5
PC (diabetic + Glibenclamide)	307±3.5	260±3	210±2.5	145±2.5
T-1 (Diabetic + Ginkgolide B)	319±2.5	270±3.5	225±2	190±2.5
T-2 (Diabetic + Ginkgolide B)	311±2.5	262±2.5	218±2.5	155±3.5

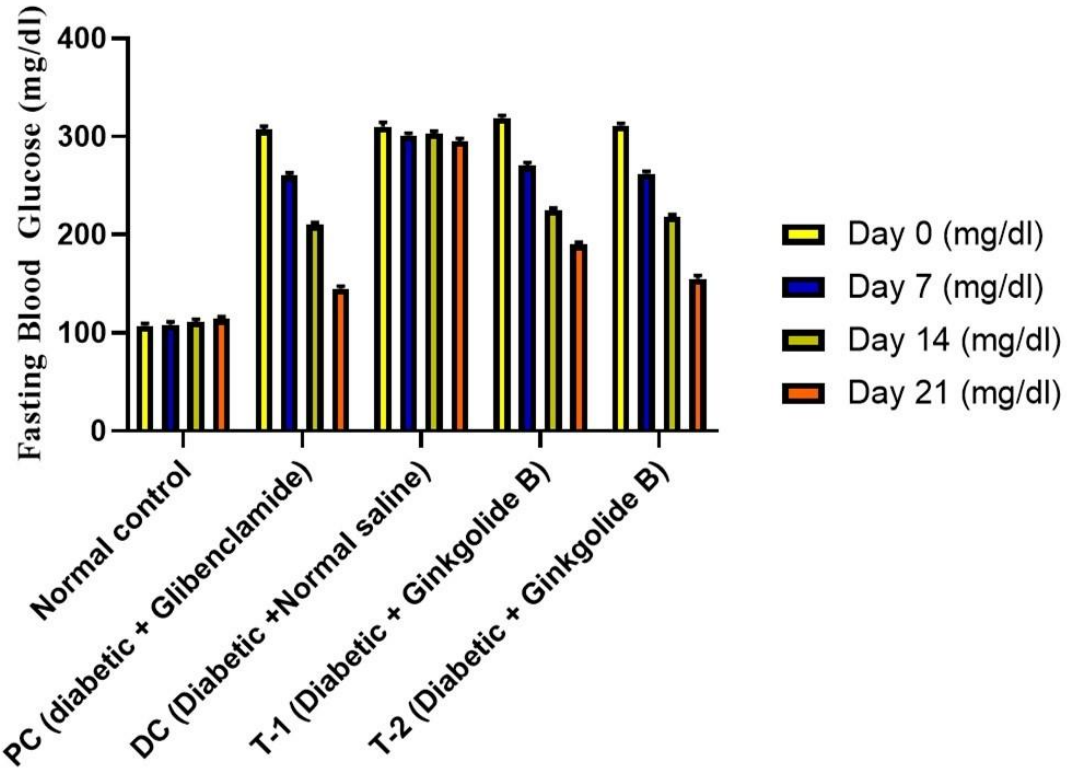


Figure 3:Fasting blood glucose levels in rats on different days.According to this graph T-2 which contain diabetic and Ginkgolide B shows extra ordinary results.

Twenty-one days later, the change of the fasting blood glucose (FBG) level of the diabetic rat was also monitored to assess the ability of the different treatments in controlling the glucose level. The rats in the Normal Control (NC) group also did not develop diabetes and the amount of glucose in their blood slightly rose from 107 mg/dl on Day 0 to 114 mg/dl on Day 21. The diabetic animals in the PC (Diabetic + Glibenclamide) group showed a significant decrease in blood glucose level after the treatment with the previously defined antidiabetic drug Glibenclamide from 307 mg/dl on Day 0 to 145 mg/dl on Day-21. This demonstrates how effectively Glibenclamide reduces hyperglycemia and bring the levels of blood glucose to normal. In the case of the glucose level of the DC (Diabetic Control + Normal saline) group, the glucose level remained high between Day 0 to Day 21 whilst the rats did not receive any treatment. C The levels of glycogen varied from 310 milligrams of chloride per decilitre to 295 milligrams of chloride per decilitre. This goes a long way in confirming the diabetic model that was used whereby diabetic rats remain hyperglycemic if not treated. Urinary FBG reduced from 319 mg/dl on Day 0 to 190 mg/dl on Day 21 in T-1 (Diabetic + Ginkgolide B) group confirm that Ginkgolide B is effective in antidiabetic activity albeit lesser than Glibenclamide. Finally, the T-2 (Diabetic + Ginkgolide B) group showed significant decline in the glucose level from 311mg/dL in Day 0 to 155mg/dL in Day 21. This group was possibly administered a different or a higher concentration and or dose of Ginkgolide B. This mean that some effectiveness of Ginkgolide B to reduce blood glucose may be dose related.

Blood lipid parameter analysis:

Table 2: Effect of NC, PC (diabetic + Glibenclamide), **DC (Diabetic +Normal saline), T-1 (Diabetic + Ginkgolide B) and T-2 (Diabetic + Ginkgolide B)** ***groups on blood lipid parameters (mg/dl) in normal control and diabetic rats***

Lipid Parameter	NC	PC	DC	T-1	T-2
Triglycerides	60±0.5	64±2	101±0.5	71±0.5	55±0.5
Total Cholesterol	72±0.5	75±1	121±2	94±0.5	79±0.5
LDL	45±0.5	39±0.5	113±0.5	110±1	98±0.5
HDL	82±0.5	80±0.5	41±1	60±0.5	71±0.5

average serum triglycerides level in mice=65 mg/dl
average serum cholesterol level in mice=26-82 mg/dl
average serum LDL in mice= Less than 100 mg/dl
average serum HDL in mice= 70–90 mg/dl

Table 2 shows the impact of different treatment on lipid profile in normal and diabetic rats. All the lipid profile values of Normal Control (NC) group inclusive of the triglyceride levels (60±0. 5 mg/dl), total cholesterol (72±0. 5 mg/dl), LDL (45±0. 5 mg/dl) and HDL (82±0. 5 mg/dl) were within the normal range for healthy mice. The triglycerides were increased and were 64±2 mg/dl higher in the PC group (Diabetic + Glibenclamide) as compared to NC group while the total cholesterol was increased and was 75±1 mg/dl. The decrease of LDL to 39±0. (toc) 5 mg/dl level and to maintain of LDL at 80±0. Sustained at 5 mg/dl near normal values means that Glibenclamide is helpful to keep lipid levels near to normal. On the other hand the Diabetic Control + Normal saline that was not treated displayed significantly higher lipid levels. Slightly higher than the average values Round off in mg/dl were triglycerides at 101 ± 0. 5, total cholesterol at 121 ± 2, and low-density lipoproteins at 113 ± 0. In untreated diabetic rats the level of HDL was reduced to 41±1mg/dl, which pointed out severe dyslipidemia. The triglycerides went down to 71±0. mg/d in the T-1 group (Diabetic + Ginkgolide B) while total cholesterol was recorded at 94 ± 0. 5 mg/dl. Together with the increase in HDL to 60±0. 5 mg/dl while LDL was relatively high at 110±1 mg/dl thus showing that Ginkgolide B has a moderate positive effect on the lipid profile in diabetic rats. Compared with other groups Diabetes took together with T-1, T-2 revealed the most significant change in lipid profile after the administration of Ginkgolide B. LDL fell to 98±0. 5 mg/dL, on normal level, HDL rose to 71±0. 15 mg/dl, similar to normal levels With regard to the authors' hypothesis, both total cholesterol and LDL cholesterol were decreased to a level closer to normal range Total cholesterol= 187± 0. 5 mg/dl and LDL cholesterol to 79±0. 5 mg/dl. Considering these results, it seems that Ginkgolide B had a positive influence on lipid profile, especially in the T-2 group and may be even more effective than Glibenclamide. Therefore, there was a drastic dyslipidemic condition in untreated diabetic rats belong to DC group while the rats treated with Ginkgolide B treated and Glibenclamide showed the beneficial effect on lipid profile. The reduction is triglyceride, cholesterol, (ldl-c) and the Raise in (hdl-c) level seen to be higher among the T-2 group.

Anti-inflammatory study:

Table 3: Summary of scores for paw edema

	Vehicle control	Standard (indomethacin)	T-1	T-2
PE at 0 min.	0.000 ± 0.000	3± 0.01	3 ± 0.01	3 ± 0.00
PE at 60 min.	0.000 ± 0.000	2 ± 0.01	2 ± 0.00 ***	2 ± 0.01 ***
PE at 120 min.	0.000 ± 0.000	1 ± 0.01	2 ± 0.00 ***	1 ± 0.01 ***
PE at 180 min.	0.000 ± 0.000	0± 0.00	1 ± 0.00 ***	0 ± 0.01***

Diabetic + Ginkgolide B (5mg /kg/day), T-2 = Diabetic + Ginkgolide B(10 mg /kg/day); Scores are Mean ± S.E.M. * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Comparison of PE scores at different time intervals for different treatment groups, namely vehicle control, standard (indomethacin), and two treatment groups T-1 and T-2 which received different concentrations of Ginkgolide B are given in Table 4. 3. As the negative or background control in the current study, the vehicle control group did not develop the paw oedema at any of the time point (PE = 0. 000 ± 0. 000), as they are never injected with an inflammatory agent. The paw oedema score of the standard group in whom indomethacin, a well known anti-inflammatory drug, was given was 3 ± 0. The highest R-squared of 0. 866 was recorded at 01: 00 minutes, and it reduced afterward. The score came down to 0 ± 0. 00 by 180 minutes, which means that inflammation is eliminated totally and showing how effectively indomethacin decreases paw oedema. At 0 minutes the average oedema score was 3 ± 0. 1 in the T-1 group (Diabetic + Ginkgolide B, 5 mg/kg/day), In general, Ginkgolide B had the same effect as the standard medicine as it improved glucose tolerance rate which was 01 in the T-1 group. Even in the experimental score, it turned to 2 ± 0. 00 (P < 0. 001) at 60 minutes meaning that this dose possessed anti-inflammatory action. Finally at the 120 minutes there was no change to the score of 2 ± 0. 00 –, which represents the decrease in inflammation than those observed in the indomethacin group. At the end of the study period 180 minutes, the oedema score reduced considerably to 1 ± 0. 00 which was even less effective compared with indomethacin. Likewise, similar to T-1, the T-2 group (Diabetic + Ginkgolide B, 10 mg/kg/day) initially performed an oedema score of 3 ± 0. 00 at 0 minutes. The score change was rather dramatic and dropped to 2 ± 0. 01 (p<0. 001) as compared to the control at 60 min indicating an anti-inflammatory action. The oedema score was reduced to 0 ± 0 by 180 minutes thus fully resolving the swelling. 01 (P < 0. 001) indicating that the effect of the extract was similar to that of the anti- spasmodic drug; indomethacin. Fecal microbiota after 120 minutes dropped to 1. 00 ± 0. 01 (p< 0·001).

Discussion

The functional groups and important molecular properties of Ginkgolide B is discussed in detail through various bond stretching and bending vibrations using FTIR analysis. Hydroxyl groups (–OH) are often found in alcohols, and can be thought of as being more intense, appearing around 3285 cm^{-1}. Ginkgolide B is a terpene lactone, and this functional group is essential for its pharmacologic action[15]. The analysis also shows a peak for carbonyl stretching at a frequency of about 1693 cm^{-1}, suggesting that the carbonyl group is likely attached to the lactone ring of ginkgolide B. This is a crucial group in the stability and reactivity of the compound, particularly in terpenoids. In addition, FTIR spectrum shows C-O stretching vibrations at 1029 cm^{-1}, indicating ether linkages that may also participate in the biological activity of the compounds. The presence of these ether bonds has a substantial impact on the solubility of the compound and potential interactions with biological targets. C-H stretching vibrations are associated with lipophilicity and interaction of the molecules with lipid membranes; we identified weak peaks at 3736 cm^{-1} corresponding to methyl (CH_3) or methylene (CH_2) groups. FT-IR spectrum analysis where

specific linkages of bond between B with peaks around 1400–1000 cm^{-1} consistent with the bicyclic structure of Ginkgolide B, shows ring vibrations & C–O, C–H bending [16]. Such characteristics fit those of a bicyclic terpene lactone, a critical compound for experimental studies of both structural characteristics as well as bioactivity potential. The antioxidant activity of Ginkgolide B was determined by a free radical scavenging method, and the results were higher than ascorbic acid. The antioxidant activity of the compound was enhanced in a dose-dependent manner showing higher scavenging activity at the elevated concentrations. The antioxidant activity of ginkgolide B was found to be 70 ± 1% at 50 µg/ml suggesting it could be a natural antioxidant. With its ability to destroy free radicals, Ginkgolide B is considered an ideal candidate for treatment of a major role in the etiology of many diseases, including cancer, neurological and cardiovascular diseases [17]. Ginkgolide B's anti-diabetic activities were compared with those of the frequently used anti-diabetic medicine, Glibenclamide. In diabetic rat models, the compound displayed a dose-dependent lowering effect of fasting blood glucose (FBG) levels, indicating it to be a promise candidate for diabetes therapy. The FBG levels decreased significantly in a pattern comparable with Glibenclamide: on the one hand, moderately decreased compared with control (C) (T-1 group received lower dose of Ginkgolide B), and on the other hand, greatly decreased (T-2 group received greater dose in comparison with C and T-1). Collectively, these findings suggest that ginkgolide B could serve as a novel complementary and alternative agent for synthetic anti-diabetic medications, particularly in patients who are not expected to tolerate conventional prescription diabetes agents. Besides its anti-diabetic effects, Ginkgolide B revealed improvement potential in lipid profiles. An increase in dose of the compound promoted improvement in triglyceride, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels in the T-2 group suggesting a lipid-lowering potency of the compound. This effect is consistent with the anti-inflammatory and antioxidant properties of the compound, known to impact lipid metabolism. The anti-inflammatory activity of Ginkgolide B was evaluated and compared with an anti-inflammatory drug, indomethacin using paw oedema model. Both fast and slow anti-inflammatory activities were observed for Ginkgo biloba extracts particularly at high and low dosage, respectively, with Ginkgolide B exhibiting both fast and slow anti-inflammatory effects, however, the activity appeared to be slower than that of Indomethacin. The T-2 group (high dose) showed early and drastic anti-inflammatory effects, reaching levels equal to indomethacin by ++grade, while the T-1 group (low-dose) showed a gradual decrease in inflammation over 24 h. These findings suggest a dose-dependent anti-inflammatory activity of ginkgolide B, which was pronounced as well as rapid at higher dosages [18]. Histopathological analysis of paw tissues also supported the anti-inflammatory effects of ginkgolide B as there was reduced oedema and inflammatory cell migration in the treated groups. The T-2 group's inflammation was totally resolved which emphasizes the compound's potential to serve as a natural alternative for nonsteroidal anti-inflammatory drugs (NSAIDs). Although its effect at low doses is delayed, ginkgolide B is an attractive therapeutic agent for diseases associated with inflammation due to its abilities to mediate oxidative stress and inflammation [19, 20]. In summary, ginkgolide B is a potent bioactive compound that has exhibited anti-inflammatory, anti-diabetic and antioxidant properties. The efficacy of PhC in reducing oxidative stress, improving glucose metabolism and modulating lipid profiles, further supports its therapeutic potential. In addition, the dose-dependent effect of Ginkgolide B, particularly at a higher dosage, suggests a view of Ginkgolide B as a natural substitute of prescription drugs, which is mainly favorable to people who prefer not to use synthetic drugs. Further research – including clinical trials – is needed to fully investigate its therapeutic applications and to maximise its appropriate dosage.

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