Antioxidant and nephroprotective potential of *Cinnamomum verum* J. Presi) bark in High Fat High Sugar Diet model coupled with LC-MS/MS analysis and formulation development

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Abstract

Medicinal plants and herbs are used from ancient times by human beings for the treatment of acute or chronic diseases because they are accessible and have low cost. In this research, we investigated preliminary phytochemical, antioxidant, nephroprotective effect of ethanolic extract of *Cinnamomun verum* J. Presi bark along with formulation development. We investigated the total phenolic content, total flavonoid content, antioxidant activity by DPPH, H_2O_2 and FRAP method. The *in-vivo* nephroprotective effect were evaluated on *Wister albino* rats using high-fat, high-sugar diet (HFHSD) model. LC-MS/MS was performed for the phytochemical profile was examined. Tablet based formulation was prepared. Results showed that total phenolic content was 175.39±3.15 mg GAE/g, total flavonoid content was 87.69±1.86 mg RE/g, DPPH scavenging potential with an $IC_{50}$was 0.60, H_2O_2 scavenging inhibition was 51.2±0.08%, and FRAP values was 65.69 µg/ g $Fe_2(SO_4)_3$ solution.The *in vivo* study showed that *Cinnamomun verum* J. Presi bark ethanolic extract significantly improved renal markers in a dose-dependent manner. LC-MS/MS data shows the presence of many phytochemicals. Pre-formulation and post formulation test were according to standards. The results of this study suggest that *Cinnamomum verum* J. Presi bark ethanolic extract can be used to treat diabetic induced renal disorders due to its antioxidant potential.

Keywords

Nephroprotective, Obesity, Antioxidant, Mass spectroscopy, Formulation.

1. INTRODUCTION

Herbal drugs have been playing substantial role in maintaining and improving the human health and quality of life from thousands of years. Application of herbal drugs is growing day by day for correcting the imbalances that were made by modern life styles and diet (Abb-

as K., *et al*.,2016). Diabetes mellitus (DM) is rapidly growing global issue and common metabolic disorder. It is most prevalent disease and affecting 10% population across the globe. Oxidative stress, triggered by elevated free radicals and reactive oxygen species play key role in the development of DM. The burden of the disease is immense owing to DM related blindness, kidney, amputation and nerve disease (Qadir, M.I., *et al.*,2013 ; Khizar, A., *et al.*, 2019). Kidney diseases which occur due to oxidative stress caused by toxic chemicals, malnutrition, medications, chemical agents and heavy metals. They adversely affect the kidneys causing acute renal failure, chronic interstitial nephritis and nephritic syndrome. This nephro toxicity can be prevented or ameliorated by use of natural anti-oxidants and plant extracts. Medicinal plants containing antioxidants, flavonoids, alkaloids, carotenoids, vitamins, glycosides and terpenoids can reduce oxidative stress. Therefore, there is an imperative need of medicinal plants used in traditional medicine have significant role in decreasing the progression of chronic kidney diseases (Abbas, K., *et al*., 2017).

Cinnamomum verum some time called as *Ceylon cinnamon* or *C. zeylanicum is a* traditional folk herb belong to Lauraceae family. It is cultivated in several Asian countries and have different varieties. Cinnamon is highly regarded for its aromatic and flavoring property. The cinnamon plant contains many active chemical constituents in its roots, bark, and leaves. Essential oil is mostly found in cinnamon bark and is composed of cinnamal dehyde, cinnamyl acetate, eugenol, â-caryophyllene, linalool, 1,8-cineole, mannitol, pentacyclicditerpenes, phenolicacids, cinnamyl alcohol, cinnamic acid, eugenol, camphor, tannin, methyl-hydroxy chalcone polymer, carbohydrates, vitamins (B3, C, K, and A), proteins and minerals (zinc, iron, phosphorous, magnesium, calcium, sodium, and manganese). Cinnamon also contains vanillic acid, gallic acid, chlorogenic acid, coumarin, dietary fiber, caffeic acid, catechol, and catechin. Cinnamon is used for cognitive enhancement, blood glucose manage-ment, cardiovascular support, also possess antioxidant, anti-microbial, anticarcino- genic and anti-inflammatory properties (Bibi, T., *et al*., 2024 ; Martolia, J. and N. *et al*., 2024). However, besi-de of all these uses, there is a strong rationale to validate the antioxidant and nephro protective potential. Therefore, the present study aims to determine the antioxidant activity of the ethanolic extract of *Cinnamomum verum* J. Presi bark using various bioanalytical methods, including ferric ($Fe^{3}z$) ion reducing abilities, hydrogen peroxide, and DPPH scavenging activities, demonstrate the extract's protective effects against obesity and diabetes induced kidney damage alongside LC-MS/MS analysis for phytochemical profiling.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

Bark of *Cinnamomum verum* J. Presi (1.0 kg) was obtained from a local market in Sahiwal, Punjab, Pakistan. The crude drug was authenticated by Professor Dr. Zafarullah Zafar from the Department of Botany at Bahauddin Zakariya University, Multan. For future reference a small sample was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan, under specimen voucher number (www.theplantlist.org/tpl1.1/record/kew-

21721692). The bark then grinded to produce a coarse powder.

2.2. Extract preparation

Five hundred grams of coarsely ground *Cinnamomum verum* J. Presi bark (CvB) powder was macerated in 1.5 liters of 98% ethanol for seven days, with occasional shaking. The soaked material was filtered using Whatman filter paper. The solid residue remaining after the first filtration was macerated again twice, first in 1.0 liter and then in 0.8 liter of 98% ethanol, and the filtrate was collected each time. All three filtrates were then combined and dried using a rotary evaporator (Buchi, Switzerland) under a pressure of 74.51 torr, at 4 rpm, and a temperature range of 30 to 40 C. The resulting thick, viscous plant extract paste was weighed to determine the percentage yield and stored in an airtight container.

2.3. Phytochemical screening

Phytochemical screening of CvB was performed for the identification of secondary and primary metabolites like glycosides, terpenoids, tannins, phenols, alkaloids, resins, flavonoids, saponins, sterols, steroids, fixed oils, proteins, gums, mucilage, and carbohydrates etc. by using the methods described by *Agarwal et. al*. 2019, *Soni et. al*. 2022 and *Yasmeen et. al.* 2023, and the results are mentioned in Table-1.

2.4. Total phenolic contents

Folin-Ciocalteu's phenolic reagent, described by *Aslam et al.* 2022, was used with slight modifications to identify the total phenolic contents (TPC) of CvB. Different concentrations of CvB solution ranging from (0.2-1 mg/mL) were prepared and 1.0 mL of each concentration were individually combined with 1.0 mL of Folin-Ciocalteu's reagent. After incubating for 5 minutes in the dark at room temperature, 10 mL of 7% Na_2CO_3 solution and 13 mL of distilled water were added to the mixture and thoroughly mixed. The reaction mixture had been incubated in the dark for an hour, the absorbance was recorded at 750 nm using UV/VIS spectrophotometer (Optima, SP-3000, Tokyo, Japan) where Gallic acid was a reference drug. The whole procedure was executed three times and the results given in milligrams of gallic acid equivalent per gram of extract (mg GAE/g) were calculated using Eq. 1. Results are mentioned in Table-2.

$$TPC = \frac{C \times DF \times V}{m} \quad \text{..... (Eq. 1)}$$

C= Concentration of gallic acid calculated from standard curve
DF= Dilution factor, V =Volume of extract
m=Weight of sample

2.5. Total flavonoids contents

The total flavonoid content found in CvB was calculated by a method prescribed by *Aslam et al.* 2022 with slight modifications. Rutin was utilized as a reference drug to calculate the total flavonoid contents. Combine 1 mL of crude extract in following range of concentrations (0.2-1 mg/mL) with 0.15 mL of $NaNO_2$ (0.5M) and 0.15 mL of $AlCl_3.6H_2O$ (0.3M). After incubating for 5 minutes, the solutions were uniformly mixed with 1 mL of 1M NaOH, followed by measuring the absorbance at 506 nm using a UV/VIS spectrophotometer (Optima, SP-3000, Tokyo, Japan). The whole procedure was executed three times and the results given in milligrams of rutin equivalent per gram of extract (mg RE/g) were calculated using Eq. 2 and are mentioned in Table-2.

$$TFC = \frac{C \times DF \times V}{m} \quad \text{..... (Eq. 2)}$$

C= Concentration of rutin calculated from standard curve
DF= Dilution factor, V =Volume extract,
m= Weight of sample

2.6. Hydrogen peroxide scavenging assay

The technique reported by *Akhter et al.* 2021 was somewhat modified in order to assess the H_2O_2 scavenging potential of CE. The H_2O_2 scavenging activity of CvB was evaluated at 0.6 mg/mL. The test solution (1 mL) from each dilution was incubated with 0.6 mL of 40 mM H_2O_2 (made in phosphate buffer at pH 7.4). After incubation for 10 minutes at room temperature in the dark, the absorbance of hydrogen peroxide at 230 nm using UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan) was measured relative to a blank solution prepared with phosphate buffer solution without hydrogen peroxide. Gallic acid served as the positive control. The entire procedure was repeated thrice and the hydrogen peroxide scavenging potential of CvB was determined using Eq. 3 and results are mentioned in Table-3.

H_2O_2 Inhibition capacity = 1 - Absorbance of sample / Absorbance of blank * 100.....(Eq. 3).

2.7. DPPH free radical scavenging assay

Diphenyl-1-picrylhydrazyl radical scavenging activity of CvB was assessed by method designated by *Wintola et al.* 2021 was adopted with small modifications. Five solutions ranging (0.2-1 mg/mL) of CvB and gallic acid were prepared in methanol. Each dilution (1mL) was mixed with 0.135 mM DPPH solution (1 mL) prepared in methanol. Then vortexed the mixture and incubated in the dark for half an hour at room temperature. The absorption of each sample was recorded spectrophotometrically at wavelength of 517 nm by using UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan) (Wintola, O.A., *et al*.,2021). The entire procedure was repeated thrice and % inhibition was calculated using Eq. 4 and the outcomes are mentioned in Table-3.

% DPPH Inhibition = Ac-As / Ac * 100 ...(Eq. 4).

Absorbance of Control = A_c, Absorbance of Sample = A_s

2.8. Ferric reducing ability of plasma (FRAP) assay

Technique described by *Benzie et al.* 1996 was executed to perfrom Ferric reducing ability of plasma (FRAP) assay.A Fresh FRAP reagent of pH 3.6 was prepared and stored at room temperature by combining 2.5 mL of 10 mmol/L TPTZ (2,4,6- tripyridyl-s-triazine, Sigma) solution in 40 mmol/L HCl with the addition of 25 mL of 0.3 mol/L acetate buffer and 2.5 mL of 20 mmol/ L $FeCl_3$. Approximately 40 ìL of the sample was diluted by adding 0.2 mL of distilled water. This mixture was then combined with 1.8 mL of FRAP reagent, and the absorbance of the reaction mixture was measured at 593 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan) after incubating for 10 minutes at 37°C. $FeSO_4$ (1 mmol/L) was served as reference standard. The final assay results were expressed as the antioxidant capacity of the CvB, measured in terms of ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$. (Benzie *et al*.,1996) The entire process is repeated thrice and results are mentioned in Table-3.

2.9. Preparation of *Wistar albino* rats

Wistar albino rats of either sex having(120±20 g in body weight) were placed in the animal house of the Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan. All rats were housed in polycarbonate cages of 7 x 34 x 18 cm^3, with a maximum of six rats per cage, 12–12 hours of light and dark, respectively and at typical temperature and humidity levels of 25±2 °C. Animals were given a conventional animal food and unlimited access to water.

2.10. *In-vivo* methodology

Animal study was performed according to standard protocol with slight modifications.

Wistar albino Rats (n=36) weighing (120±20 g) was habituated for 14 days prior to start of experiment then all the groups are continued with listed treatments.

Group I: It was called as normal control (NC) and consists of 6 animals, receives normal saline at the rate of 5 mL/kg of body weight per-oral for complete period of study.

Group II: Consists of 30 rats, receives 3 ml/kg body weight of High Fat High Sugar Diet (HFHSD) that composed of Vanaspati ghee and coconut oil (3:1) and 10 mL/kg 25% dextrose water P.O. for 56 days. Then these animals were treated with streptozotocin (65 mg/kg) interperitoneally prepared in pH 4.5 citrate buffer. Blood glucose level was checked after 48 hours after injection. Animals showing blood glucose level e"200 mg/mL were arbitrarily split up into following 5 groups C-G (n=6) and were fed with HFHSD for complete period of study (84 days).

Group II (A): It was called as Obesity-Diabetes Control (ODC) group and was administered normal saline orally 5 mL/kg of body weight for remaining period of 28 days.

Group II (B): It was called as Obesity-Diabetes Treatment (ODT) group, rats in this group were administered with Glibenclamide (10 mg/kg) and atorvastatin (60 mg/kg) for remaining period of 28 days.

Group II (C): It was called as Obesity-Diabetes Treatment (ODCvB1) group in which rats were fed with CvB (100 mg/kg) orally for remaining period of 28 days.

Group II (D): Obesity-Diabetes Treatment (ODCvB2) group, in this group rats were provided with CvB (250 mg/kg) for a period of remaining period of 28 days.

Group II (E): Obesity-Diabetes Treatment (ODCvB3) group in which rats were given with CvB (500 mg/kg) for remaining period of 28 days (Munshi *et al*., 2014 ; Gomez Dominguez, E., *et al*., 2006). Blood samples for RFTs (Creatinine and blood urea nitrogen) were collected by closed cardiac puncture at start, after 56 days of HFHSD and after 84 days' study period and preserved at -20 C.

2.11. Statistical analysis

Every result was revealed as the mean of the standard error of n=6 for every set, GraphPad Prism 9.0.1 (La Jolla, CA, USA) was used for statistical evaluation for determining the results by two-way ANOVA and Tukey's test, which was used to determine the significance between different groups by setting Pd"0.05.

2.12. Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LCMS/MS) Analysis

LC-MS/MS analysis was performed on an ion trap (IT) tandem mass spectrometer (AmaZon speed, Bruker Daltonics, Bremen, Germany) hyphenated with a Thermo Fischer Scientific U-

UHPLC system (Bremen, Germany). 10 ìL of 1 mg/mL CvB was introduced into the Agilent Technologies Zorbax SB-C18 column (2.1 mm x 50 mm, 1.8 m, CA, USA) via auto sampler for a gradient separation. Separation was achieved using a flow rate of 0.2 mL/min of eluent A (0.1% formic acid in water) and eluent B (0.1% formic acid in methanol), changing from 5% B to 100% B in 20 minutes. 100% B was maintained for 5 minutes before returning to 5% B. Compounds were ionized using ESI ion source operation in positive ion mode at 4500 V. Other ion source parameters were as follows: dry gas pressure was 10 psi, dry gas flow was 4 L/min, and dry gas temperature was 180 °C. The mass scan range was 100 to 2000 amu. The top 10 precursor ions based on intensities were selected and fragmented in an automatic fashion. The MS/MS scan range was 50–2000 amu. MS/MS data was processed using Compass Data Analysis 4.4 (Bruker Daltonics). All MS/MS spectra were converted to mgf format. Mgf files were then searched in Global Natural Product Social Molecular Networking (GNPS) Libraries Search using default search parameters, i.e., MS tolerance of 02 amu, MS/MS tolerance of 0.5 amu with at least 3 peaks matching, and a score threshold of 0.8 MS/MS matching was then manually validated (Zeng, G., *et al*., 2006). Results of LC/MS analysis are mentioned in Table-4.

2.13. Preparation of tablet-based formulation

Cinnamon verum extract 500 mg was mixed with 200mg of starch and 100mg of lactose in a mixer. Then 30 mg of PVP-K30 and talc 5 mg was added respectively with continuous mixing to attain uniform mixture. After that 6 mg of aerosil and magnesium stearate (4 mg) was added and mixed-up. Granules of the mixture were prepared and different tests of pre formulation study was performed. Then these granules were pressed in single punch machine.

2.14. Pre-formulation study

The waft characteristics of the combined powder should be studied carefully since they affect the dosage uniformity. Before compression following parameters were tested (Kumar, T.S., *et al*., 2021).

2.15. Bulk density

The bulk density of the granules was measured by pouring the granules of weighed quantity to the graduated cylinder. Bulk density of formulated granules was calculated by using following formula:

Bulk density (V_o) = mass of granules / Volume of granules in graduated cylinder ....(Eq. 5).

2.16. Tapped density

The tapped density parameter is carried out using a density tester device. The density tester's graduated cylinder is filled with a known mass of powder and then secured to the equipment. The testing device is tapped for 100 taps from a set height of 14mm and another 3mm on the surface of the density tester, or until a consistent volume is detected, and then the tapped density is computed by:

Tapped density (V_i) = mass of granules / Volume of granules in graduated cylinder after tapping (ml) ...(Eq. 6).

2.17. Hausner's ratio

The ratio of tapped density and bulk density is Hausner's ratio. An Ideal range is ought to be in

between1.2-1.5.
Hausner's ratio = Tapped density / untapped density ...(Eq. 7).

2.18. Compressibility index

Compressibility index of formulation granules was calculated using following formula;

Compressibility index = untapped density - Tapped density / untapped density * 100 ..(Eq. 8).

2.19. Angle of repose

The tangent of the attitude of repose is the same as the coefficient of friction between the particles. As a result, the rougher and more irregular the particle floor, the greater the possibility of repose. For the dedication of attitude of repose (θ), the mixture was poured through the walls of a funnel that was held in place so that its lower tip was precisely 2.0 cm above a hard floor. The mixture was poured until the top tip of the pile floor reached the bottom tip of the funnel. The following equation is used to compute the angle of repose.

$\tan\theta$ = height(h) / radius(r) ...(Eq. 9).

The waft properties of the granules may be classified using the Angle of repose, Compressibility index, and Hausner's ratio (Podczeck *et al*., 2004 ; Leon Lachman *et al*., 1991).

2.20. Tests of prepared tablets

After measuring waft characteristics, the granules of all six batches were subjected to compression using single punch tablet compression machine. All the batches were then checked subjected to physical evaluation tests for tablets i.e. appearance, weight variation, hardness, diameter, thickness, friability and disintegration time.

2.21. Physical appearance

Tablets were evaluated for look by examining 20 tablets each batch and visually inspected the tablet's core surface for any discolorations and surface roughness (Modasiya, M., *et al*.,2009).

2.22. Weight variation test

The weight of tablets was measured by individually weighing 20 tablets and calculating the average weight. If two or more of the single weights vary from the 7.5 percent percentile limit, the tablets will pass the test.

2.23. Hardness test

Monsanto's hardness tester was used to make tablets tough, and the hardness in kg/cm^2 units was reported (Modasiya, M., *et al*., 2009).

2.24. Diameter and thickness

Vernier-calipers were used to measure the tablets' thickness and diameter, and their measurements in millimeters (mm) were recorded.

2.25. Friability

Electro laboratory friability instruments have been used to measure the friability of tablets. The 20 pre-weighed tablets were evaluated using a 25 rpm plastic chamber that drops the tablets six inches away with each operation over the course of 100 revolutions, and they were timed in friction apparatus to test for the consequences of abrasion and shaking (Anand *et al*., 2013). The following formula was used to compute friability of tablets.

Friability = Initial weights of Tablets - Final weights of Tablets / Initial weights of tablets * 100....(Eq. 10).

2.26. Disintegration time

The disintegration instrument was used to evaluate six tablets. Six tablets were placed in a basket comprising of six glass tubes with six discs placed in 900 ml of water heated to 37 °C in a 1000 ml beaker. Disintegration time is the amount of time required for the tablet to properly disintegrate (Pharmacopoeia, I., *Govt. of India., 2007.*

3. RESULTS AND DISCUSSION

Cinnamomum verum J. Presi Bark extract (CvB) when undergone preliminary phytochemical analysis resulted the presences of certain phytochemicals mentioned in Table-1, while total phenolic, total flavonoid results are mention in table-2, hydrogen peroxide, DPPH and ferric reducing power results are shown in Table-3. LC-MS/MS profile of CvBis presented in Table 4. Results of effect of CvB on renal parameters like creatinine levels and blood urea nitrogen are shown in Figure-1 and 02.

Table 1. Phytochemical screening of *Cinnamomum verum* bark ethanolic extract

S. No.	Phytochemical constituents	Test	CvB
1	Tannins & Phenols	Lead acetate Test	+
		Ferric Chloride Test	+
		Gelatin Test	+
2	Terpenoids	Salkwoski's Test	+
3	Glycosides	Borntrager's Test	-
		Keller Killiani Test	-
4	Alkaloids	Hager's Test	+
		Drangendrof's Test	+
		Wagner's Test	+
		Mayer's Test	+
5	Flavonoids	Lead acetate Test	+
		Alkaline Reagent Test	+
6	Steroid	Salkwoski's Test	+
7	Sterol	Salkwoski's Test	+
8	Proteins	Ninhydrin Test	-
		Biuret Test	-
9	Carbohydrates	Molisch's Test	-
		Benedict's Test	-
		Barford's Test	-

*Where (+) sign indicates presence and (–) sign shows the absence of phytochemical constituent

Table 2. Total phenolic and flavonoid contents of *Cinnamomum verum* bark extract (CvB)

S.No.	Parameter	Plant extract	Standard
1	TPC	175.39±3.15 mg of GAE/g	186±1.23 mg of GAE/g
2	TFC	87.69±1.86 mgRE/g	99± 0.43 mgRE/g

*TPC: Total Phenolic Content; TFC: Total flavonoids Content

Table 3. Antioxidant potential of *Cinnamomum verum* bark extract (CvB)

S.No	Parameter	Plant extract	Standard
1	H_2O_2 Assay	51.2±0.08 % inhibition	71.2±0.008 % inhibition
2	DPPH Assay	0.60 IC_{50} mg/mL	0.013 IC_{50} mg/mL
3	FRAP Assay	65.69 IC_{50} (µg/g of $FeSO_4$)	-

*H_2O_2: Hydrogen peroxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing ability of Plasma

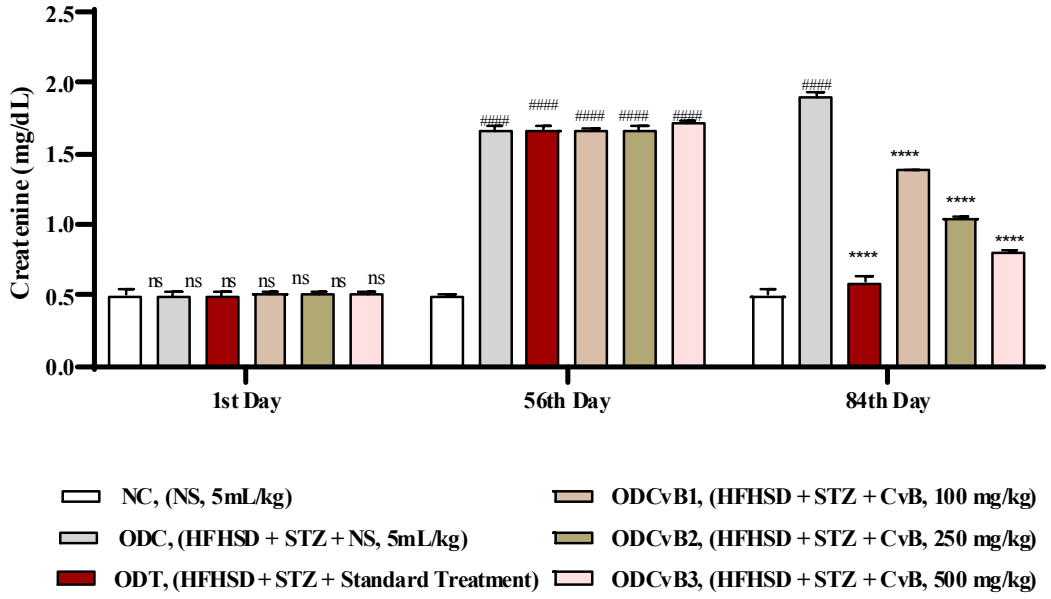

Fig-1: Changes in creatinine levels of *Wistar albino* rats STZ was administered via the intraperitoneal route after obesity induction in rats by feeding with HFHSD for 56 days followed by atorvastatin 60 mg/Kg bwt, glibenclamide 10 mg/Kg bwt and CvB doses at (100, 250 and 500) mg/Kg bwt along with HFHSD for 28 days treatment period. Creatinine levels of rats were measured with commercial kit method. Each group (n = 6) has its mean ± standard error of mean (SEM) is examined using two-way ANOVA and Tukey's post hoc test. At 1st day all the groups showed non-significant (ns) variations as compared to normal control (NC) group. After 56 days' period,

all the groups exhibited p<0.001: very significant (###) while comparing to normal control (NC) group. After 58 days' period, all the groups exhibited p<0.001: very significant denoted by (###) while comparing to normal control (NC) group. After 84 days' period, when the obesity control (OC) group was compared with obesity treatment (OT) group and obesity diabetes control (ODC) group is compared to a treatment groups (ODT, ODCvB1, ODCvB2 and ODCvB3), results exhibiting p < 0.0001: highly significant denoted by (****) p<0.001: very significant denoted by (***), p<0.01 more significant denoted by (**) ("d), p<0.05: significant denoted by (*), and p>0.05 are considered non-significant denoted by (ns). The NC group while compared with OC group and ODC group, p < 0.001: very significant results are denoted with (###).

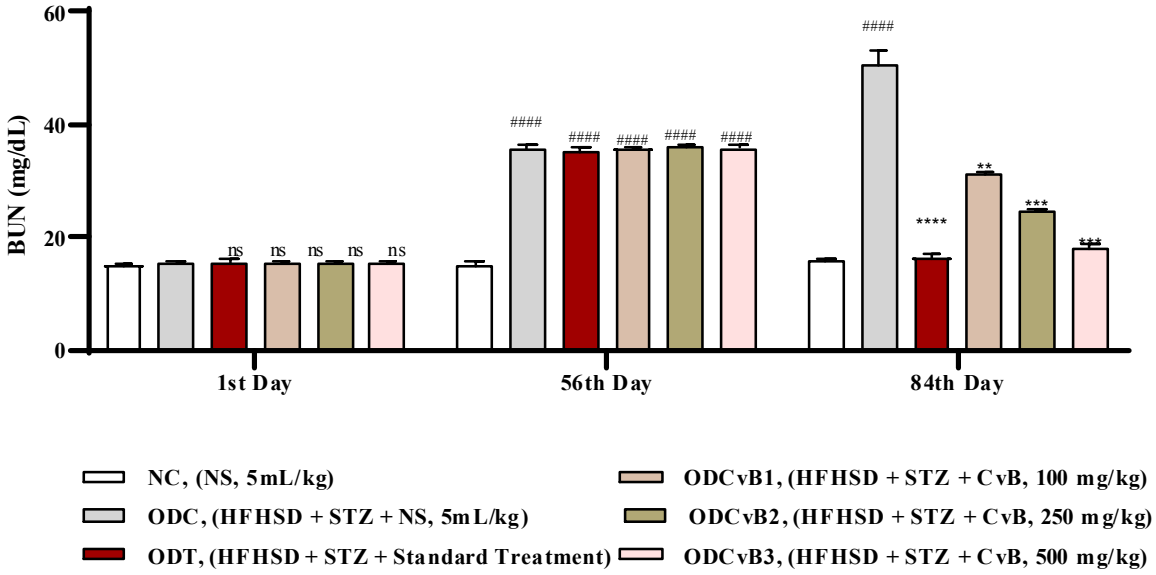

Fig-02: Changes in blood urea nitrogen (BUN) level of Wistar rats

STZ was administered via the intraperitoneal route after obesity induction in rats by feeding with HFHSD for 56 days followed by atorvastatin 60 mg/Kg bwt, glibenclamide 10 mg/Kg bwt and CvB doses at (100, 250 and 500) mg/Kg bwt along with HFHSD for 28 days treatment period. BUN levels of rats were measured with commercial kit method. Each group (n = 6) has its mean ± standard error of mean (SEM) is examined using two-way ANOVA using Tukey's post hoc test. At 1st day all the groups showed non-significant (ns) variations while comparing to normal control NC group. After 56 days' period, all the groups exhibited p<0.001: very significant (###) while comparing to normal control (NC) group. After 58 days' period, all the groups exhibited p<0.001: very significant denoted by (###) while comparing to normal control (NC) group. After 84 days' period, when the obesity control (OC) group was compared with obesity treatment (OT) group and obesity diabetes control (ODC) group is compared to treatment groups (ODT, ODCvB1, ODCvB2 and ODCvB3), results exhibiting p < 0.0001: highly significant denoted by (****) p<0.001: very significant denoted by (***), p<0.01 more significant denoted by (**), p<0.05:

significant denoted by (*), and p>0.05 are considered non-significant denoted by (ns). The NC group while compared with OC group and ODC group, p < 0.001: very significant results are denoted with (###).

Table 4. LC-MS/MS profile of *Cinnamomum verum* bark extract (CvB)

Sr.#	Name of Compound	Molecular Formula	Molecular weight (g/mol)	m/Z	Retention Time (min)
1	trans-Cinnamaldehyde	C_9H_8O	132.16	133.065	0.8
2	Squamatic acid	$C_{19}H_{18}O_9$	390.34	391.085	1.3
3	Xanthyletin	$C_{14}H_{12}O_3$	228.24	229.086	1.8
4	Piceatannol	$C_{14}H_{12}O_4$	244.24	245.11	1.9
5	Indoleacetic acid	$C_{10}H_9NO_2$	175.18	176.071	2.2
6	Kavain	$C_{14}H_{14}O_3$	230.26	231.096	2.35
7	Caffeine	C8 H1 0 N4 O2	194.19	195.087	2.01
8	Malic acid	$C_4H_6O_5$	134.08	135.03	2.7
9	Miltirone	$C_{19}H_{22}O_2$	282.38	283	2.8
10	p-methoxycinnamic acid ethyl ester	$C_{12}H_{14}O_3$	206.24	207.1	2.9
11	L-Tyrosine	$C_9H_{11}NO_3$	181.19	182.05	3.2
12	N-acetyl-DL-serine	C_5H_9NO	147.13	148.12	4.3
13	Oxolinic acid	$C_{13}H_{11}NO_5$	261.23	262.071	4.6
14	Indole-3-aldehyde	C_9H_7NO	145.16	144.045	6.6
15	Thymidine	$C_{10}H_{14}N_2O_5$	242.22	243.1	7.0
16	1-Naphthylamine	$C_{10}H_9N$	143.19	144.081	7.2
17	Psoralen	$C_{11}H_6O_3$	186.16	187.04	7.7
18	Magnoflorine	$C_{20}H_{24}NO_4^+$	342.41	342	8.6
19	Miltirone	$C_{19}H_{22}O_2$	282.38	283	8.7
20	Xanthotoxin	$C_{12}H_8O_4$	216.19	216.984	9.2

21	2-Methoxy-1-naphthaldehyde	$C_{12}H_{10}O_2$	186.21	187.075	9.5
22	Coumarin	$C_9H_6O_2$	146.14	147.149	10.35
23	Tocotrienol	$C_{28}H_{42}O_2$	410.63	411.1	10.5
24	cis-7-Hexadecenoic acid methyl ester	$C_{17}H_{32}O_2$	268.40	269.248	10.6
25	Daphnin	$C_{15}H_{16}O_9$	340.28	341.087	10.9
26	Mephedrone	$C_{11}H_{15}NO$	177.24	178.123	10.85
27	Esculin	$C_{15}H_{16}O_9$	340.28	341.086	12.1
28	Dihydroartemisinin	$C_{15}H_{24}O_5$	284.35	285.092	13.6
29	Eugenol	$C_{10}H_{12}O_2$	164.20	165.092	14.2
30	Indole-3-Carboxaldehyde	C_9H_7NO	145.16	146.06	14.3
31	Indole-3-carboxylic acid	$C_9H_7NO_2$	161.16	162.055	14.4
32	indole-3-acetaldehyde	$C_{10}H_9NO$	159.18	160.076	15.3
33	Tartaric acid	$C_4H_6O_6$	150.08	151.01	16.1
34	Piperine	$C_{17}H_{19}NO_3$	285.35	286.144	16.9
35	cis-7,10,13,16-Docosatetraenoic acid	$C_{22}H_{36}O_2$	332.5	333.195	17.15
36	Ellagic acid	$C_{14}H_6O_8$	302.19	303.01	18.3
37	7-(Dimethylamino)-4-methylcoumarin	$C_{12}H_{13}NO_2$	203.24	204.102	18.6
38	Indoxyl Sulfate	$C_8H_7NO_4S$	213.21	214.06	18.9
39	Indole-3-butyric acid	$C_{12}H_{13}NO_2$	203.24	204.102	19.0
40	Stearidonic acid	$C_{18}H_{28}O_2$	276.4	277.23	20.7

41	trans-Zeatin	$C_{10}H_{13}N_5O$	219.24	220	20.8
42	Monoelaidin	$C_{21}H_{40}O_4$	356.54	357.29	22.1
43	cis-Parinaric acid	$C_{18}H_{28}O_2$	276.4137	277.215	22.3
44	Pinolenic acid	$C_{18}H_{30}O_2$	278.42	279.21	22.4
45	Pyropheophytin a	$C_{53}H_{70}CuN_4O_3$	874.69	875.567	26.9
46	Oleic acid ethyl ester	$C_{20}H_{38}O_2$	310.51	311.29	28.9
47	13-Docosenamide	$C_{22}H_{43}NO$	337.58	338.343	29.0

Table 5. Pre-formulation data of *Cinnamomum verum* bark extract (CvB) granules

Bulk density (V_o)	Tapped density (V_i)	Hausner's ratio	Compressibility index	Angle of repose
0.54	0.73	1.35	35.179	33.04

Table 6. Physio-chemical parameter of prepared tablets

Physio-chemical parameter	Observation
Shape	Round tablets, Smooth surface
Color	Dark green
Odour	Characteristic odor
Taste	Bitter
Moisture content (%)	3.42%

Table 7. Quality control tests of *Cinnamon verum* tablets

Weight variation (%)	Thickness (mm)	Diameter (mm)	Hardness (Kg/cm^2)	Friability (%)	Disintegration time (min)
845±2.17	5.41±0.03	17.41±0.13	10.50±0.25	0.57	8.19

Medicinal plants are being revisited for diabetes treatment, with many conventional drugs derived from their compounds; for instance, metformin, an effective oral hypoglycemic agent, was developed from guanidine found in *Galega officinalis* (Witters, L.A., *et al*., 2001). Over 400 traditional plant treatments for diabetes have been reported, but few have undergone scientific and medical

evaluation for efficacy. Despite the availability and regular use of many herbal formulations by diabetic patients, the major obstacle to their acceptance in allopathic medicine is the lack of scientific and clinical data on their efficacy and safety (Dham, S., *et al*.,2006). Clinical research is needed to evaluate the pharmacological and toxicological profiles of these herbal drugs, develop animal models for toxicity and safety assessment, and identify active components to develop effective medications (Modak, M., *et al*., 2007). In vivo benefits of *Cinnamomum verum* include attenuated diabetes-associated weight loss, reduced fasting blood glucose, lowered LDL and HbA1c, increased HDL cholesterol and circulating insulin, and improved metabolic derangements linked to insulin resistance (Ariyanto, E.F., *et al*., 2021). In current research the phytochemical, anti-oxidant and renal protective efficacy of a formulation prepared from *Cinnamomum verum* J. Presi bark extract was assessed using *in vivo* diagnostic markers.

In this study, the exploration of phytochemicals using phytochemical tests on CvB has revealed the presence of many vital metabolites in the *Cinnamomum verum* J. Presi Bark. The phytochemicals found include tannins, phenols, terpenoids, alkaloids, resins, flavonoids, steroid, sterol, and fixed oils.The biological activity of these compounds plays a crucial role in contributing to their pharmacological and physiological effects (Lakshmi, V.J. *et al*., 2021).

Phytochemicals, such as flavonoids and phenolic compounds, found in nutrients and herbal medicines, are effective antioxidants, antimicrobial, anticancer, anti-inflammatory agents, and cardio protective, they promote the immune system and protect the skin from UV radiation, making them valuable for pharmaceutical and medical applications (Tungmunnithum, D., *et al*.,2018). Phenolic compounds, derived from the aromatic amino acids phenylalanine and tyrosine as secondary metabolites, are biologically significant due to their hydroxyl groups, which grant them scavenging capabilities and a substantial role in various physiological processes (Sarian, M.N., *et al*., 2007). In the current study, the phenolic and flavonoids content in CvB was 175.39 ± 3.15 and87.69±1.86 respectively. To comprehensively evaluate the antioxidant capacity of CE, our study employs three assays—DPPH radical scavenging, hydrogen peroxide scavenging, and FRAP—recognizing that a single model cannot fully capture the diverse mechanisms of antioxidant action. The DPPH free radical scavenging assay is widely used to evaluate the antioxidant potential of plant extracts and compounds, with the lowest IC_{50} indicating the strongest antioxidant activity (Pratap Singh, R. and A.K. *et al*., 2023). The DPPH radical scavenging assay revealed that CvB has lower IC_{50} value of 0.60, indicating significant antioxidant activity through hydrogen donation to stabilize free radicals (Sekhon-Loodu, S. and H.V., 2019). However, gallic acid, the positive control, demonstrated superior antioxidant potential with an IC_{50} value of 0.013. In the present study, CvB efficiently scavenged 51.2 ± 0.08% of hydrogen peroxide, suggesting that phenolic groups within the extract neutralize H, O, into water by donating electrons. These phenolic compounds, known for their electron-donating properties, likely contributed to this process, preventing the formation of hydroxyl radicals and subsequent lipid peroxidation. This activity underscores the extract's potential to mitigate oxidative stress, highlighting its relevance in antioxidant applications (Oyedemi, S.O *et al*., 2010). This activity underscores potential of CvB

to mitigate oxidative stress, highlighting its relevance in antioxidant applications. Mounting evidence indicates that human obesity is a state of chronic oxidative stress, characterized by an imbalance between tissue free radicals, reactive oxygen species (ROS), and antioxidants, which may be a key mechanism underlying obesity-related co morbidities (Vincent *et al*., 2007). The FRAP assay, based on electron transfer rather than hydrogen ion transfer, was employed in our study to determine the antioxidant potential of the extract. This assay measures the reduction of $Fe^{3}z$ to $Fe^{2}z$, producing a colored complex with $Fe^{2}z$. The reaction involves the reduction of the Fe(III)-TPTZ complex to the blue-colored Fe(II)-TPTZ by an antioxidant agent, with increased absorbance indicating higher reducing power in this method. The strength of this reduction is directly related to the degree of hydroxylation, indicating that higher phenolic and flavonoid content results in a lower IC_{50} value in the FRAP assay (Asem, N., *et al*.,2020). In our research, the IC_{50} of CvB was 65.69 µg/g of $FeSO_4$, shows that CvB contains moderate levels of phenols and flavonoids content in sample indicating significant antioxidant potential of CvB therefore it can be used to reduce obesity and diabetes induced complications (Hassan, W., *et al*., 2014).

In our research, we developed an obesity-diabetes model in rats by feeding them a high-fat high-sugar diet for 8 weeks and administering a single dose of streptozotocin (STZ). This model showed noticeably higher fasting blood glucose levels compared to the rats in the normal control group (Gomez *et al*., 2006 ; Sreejesh *et al*., 2017). Throughout the study, we carefully tracked body weights, noting a substantial increase in the obesity-diabetes model (ODC) group. This indicates successful obesity development in the model, marked by increased adipose tissue mass and accompanying metabolic abnormalities like elevated levels of glucose, leptin, or insulin. These findings emphasize the relevance and credibility of our established model in simulating the intricate relationship between obesity and diabetes (Collins, K.*et al*.,2018). Serum Creatinine level showed a noteworthy elevation ($p < 0.005$) in OC and ODC groups. After treatment with CvB (100, 250 and 500 mg/Kg) a noteworthy decline ($p< 0.005$) in creatinine level was noticed in ODCvB1, ODCvB2 and ODCvB3depending upon the dose as shown inFigure-1.Blood urea nitrogen (BUN) level demonstrated a significant elevation ($p < 0.005$) in OC and ODC groups. After treatment with CvB (100, 250, and 500 mg/kg), a considerable decrease in BUN levels ($p < 0.005$) was observed in a dose-dependent manner across the different groups (ODCvB1, ODCvB2 and ODCvB3) compared to the ODC group, as shown in Figure-2.

The ethanolic extract of *Cinnamomum verum* J. Presi (CvB) was profiled and evaluated using LC-MS/MS in both negative and positive ionization modes for the qualitative characterization of its constituents.Characterization of compounds was carried out by absorption spectrum in the UV-visible region, retention times, spectrum obtained by fragmentation profile, MS-ESI, and with comparison with the aforementioned literature (Table-4). LC-MS/MS analysis results facilitated the tentative assignment of components using positive ionization mode. In the present research work, the positive mode ESI was more sensitive for the identification of alkaloids, flavonoids, terpenoids and coumarins in the extract.Many anti-obesity and anti-diabetic drugs currently on the market have inconsistent efficacy and come with notable side effects. This has led to a growing interest in herbal and Ayurvedic folrmulations.

These natural drug formulations are gaining popularity as treatments for metabolic disorders like diabetes and obesity. *in vivo* studies have indicated that CvB contains promising anti-obesity and anti-diabetic compounds, suggesting that a tablet-based formulation of CvB could be a viable alternative to conventional allopathic medications for these conditions. Based on the *in vivo* profile, CvB 500 mg tablets were developed and the granules were analyzed for compressibility index, angle of repose, tapped density, Hausner's ratio, and bulk density, all of which were within the acceptable range. Further studies on the compressed tablets assessed hardness, weight variation, disintegration time, and friability. The total weight of the tablets varied within the acceptable limit of ±5%. The tablets showed a friability of less than 5%, uniform thickness, and satisfactory hardness. Additionally, the disintegration tests met the required criteria.

4. CONCLUSION

It is concluded from the current research work that ethanolic extract *Cinnamomum verum* J. Presi (CvB) posses *in vitro* antioxidant, renal protective effect. We have developed a readily soluble formulation for human consumption that effectively manages hyperglycemia in obese diabetic individuals.

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Ethical approval and consent to participate:

The Institutional Animal Ethics Committee, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, approved the experiment's protocol, and the experiment was conducted in compliance with the Panel's rules for the monitoring and administration of animal experiments underInstitutional Bioethical Committee Approval No. IBC KU-289/2022.

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The authors declare that they have no conflict of interest.

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