



Evaluation of Antihypertensive efficacy of *Trapa natans* Peel extract in Fructose Induced Hypertension

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Abstract

Cardiovascular illness is one of the core reasons of fatality worldwide. Hypertension affects more than 1.3 billion people globally and is the most common underlying cause for cardiovascular disease and mortality. The main objective of this study was to explore the effect of methanolic extract of *Trapa natans* peel on fructose induced hypertension in rat model. The experiment included Wistar albino rats ($n=24$) weighing (150-180gram), including negative control group ($n=6$) which was fed with standard chow diet, while the rest of rats ($n=18$) fed with 10% fructose daily for 6 weeks. After the confirmation of hypertension, rats were classified into three groups, positive control group (hypertensive rats), standard group (treated with Amlodipine (5mg/kg/bwt) and treatment group methanolic extract of *Trapa natans* peel 200mg/kg orally) daily for two weeks. Decapitation was performed on 7th and 14th day of treatment to collect blood samples for serum biochemical analysis (oxidative stress markers, liver profile, lipid profile, electrolytes and RFTs) and tissues of heart, kidney and aorta for histological examination. The heart tissues were used for gene expression of the ACE, iNOS, eNOS, COX2 and NFkB through qRT-PCR. Systolic blood pressure (SBP) 171mmHg was significantly ($P\leq 0.05$) decreased to 130mmHg and diastolic blood pressure (DBP) 120mmHg was significantly ($P\leq 0.05$) decreased to 84mmHg in treatment group in comparison to positive control group. The total oxidant status, serum malondialdehyde, low-density lipoproteins, serum triglycerides, serum cholesterol, AST, ALT, serum uric acid, creatinine, serum sodium level and heart rate were decreased significantly ($P\leq 0.05$) in treatment group in comparison to positive control group. While there was significant ($P\leq 0.05$) increase in total antioxidant capacity, high-density lipoproteins and serum potassium level as compared to positive control. Histological and ECG changes confirm the significant improvement in heart rate and resorting the myocardial alterations. The current study showed promising protective efficacy of *Trapa natans* peel by improving the structural changes in cardiomyocyte due to its significant antihypertensive potential.

Introduction

Cardiovascular illness is a main cause of fatality worldwide. Majority of deaths from cardiovascular disease or stroke are related to hypertension (which is the world's leading risk factor). Elevated blood pressure (BP) is directly linked to a number of negative consequences, such as heart and vascular disease and renal diseases (Mills et al., 2020). The

number of people with persistent high blood pressure is anticipated to increase from 118 million in 2000 to 214 million in 2025, which includes approximate number of both genders, including men and women (Mishra et al., 2020). The latest hypertension guidelines from the American College of Cardiology and American Heart Association in 2017 was the normal range of blood pressure is (SBP ≤

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120mmHg or DBP $\leq$ 80mmHg), elevated (SBP between 120-129mmHg and DBP less than 80mmHg), stage 1 hypertension (SBP between 130-139mmHg or DBP between 80-89mmHg), and stage 2 hypertension (SBP at least 140mmHg or DBP at least 90mmHg) stated in (Bhat et al., 2023).

Age, high body mass index (BMI), eating too much salt, smoking, living a sedentary lifestyle, and drinking too much alcohol are all significant risk factors for hypertension (Meher et al., 2023). Renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system activation, inflammatory mediators, restricted capillary blood flow, and endothelial dysfunction are one of the variables that contribute to the physiopathology of persistent high blood pressure (Harrison et al., 2021). The renin-angiotensin-aldosterone system (RAAS) controls metabolism and is crucial for maintaining bodily fluid balance, cardiovascular homeostasis, and electrolyte balance. There is evidence that the RAAS is crucial for the development of hypertension (Clafin et al., 2017).

Mechanisms behind modifications in vascular shape and function brought on by oxidative stress. Reactive oxygen species in the vasculature produce extracellular matrix changes, enhanced vascular cell migration and proliferation, apoptosis, inflammation, and other processes that may finally result in vascular remodeling and hypertrophy. In addition, ROS impairs endothelial function and reduces the bioavailability of vascular nitric oxide. All of these alterations aid in the structural and operative alterations of the vascular system that are connected to the emergence and maintenance of hypertension (Shaito et al., 2022).

Endothelial dysfunction is one of the primary pathways known to contribute to oxidative stress. Increased oxidative stress may be the cause of NO metabolism disturbances, which may be due to higher NO degradation, NO inactivation, or presence of NO inhibitors (Maamoun et al., 2019). When treating hypertension, a combination of medicines is commonly used (Flack & Adekola, 2020). ACE inhibitor and a calcium channel blocker is one of the best blood pressure medication combinations. Even medications that are successful in lowering high blood pressure have unpleasant and perhaps harmful side effects (Gill et al., 2019). Due to their low cost and ease of access, medical herbs are an essential component of traditional medicine. These may be used with other treatments to prevent or cure hypertension and other cardiovascular diseases (Al Disi et al., 2016).

The aquatic floating plant *T. natans*, often known as the water chestnut or water caltrop, is indigenous to Eurasia, Africa, and Asia. The plant known as water caltrop is very helpful in the Indian Ayurveda medical system, and it is a significant meal called "singhara". This specific fruit has several health benefits, including anti-inflammatory, anti-diabetic, anti-

microbial, immunomodulatory, neuroprotective, and anti-ulcer action, among many others (Rajput & Singh, 2023). Investigating the potential applications of *Trapa natans* aquatic plant, particularly the peels, which are currently considered waste, may lead to the identification of a source of food, energy, or medicine that is commercially feasible (Chiang & Ciou, 2010). An analysis of the chemical composition of *T. natans* fruits revealed that they may be significant sources of minerals, proteins, and carbohydrates that are acceptable for dietary inclusion (Samantaray et al., 2021). The exact molecular mechanism by which *T. natans* affects the cardiovascular system and, in particular, on important genes controlling blood pressure (such as *ACE*, *COX-2*, and *eNOS*) was still entirely unknown, despite these encouraging preventive properties. Its therapeutic translation is limited by this lack of mechanistic understanding. Therefore, the main objective of our study was to evaluate the antihypertensive potential of methanolic extract of *T. natans* by examining the impact of *T. natans* on the gene expression profile of *ACE*, *COX-2*, and *eNOS* to clarify its precise mechanism as an antihypertensive drug, as this study sought to expand existing understanding.

Materials and Methods

Experimental design

The experiment involved *Wistar albino* rats (n=24) weighing (150-180gram) randomized into negative control group (n=6) which was fed with standard chow diet, while the rest of rats (n=18) fed with 10% fructose daily for 6 weeks. After the confirmation of hypertension, rats were classified into three groups, positive control group (hypertensive rats), standard group (treated with Amlodipine (5mg/kg/bwt) and treatment group methanolic extract of *T. natans* peel 200mg/kg orally daily for two weeks (Banu et al., 2023).

Procurement of plant and extract formation

T. natans were bought from the local market in Faisalabad, and the peels were separated and dried in the shade for four weeks. Using a mechanical grinder, the plant material was ground into a powder and then mixed in methanol for four days at room temperature. A Soxhlet device was used to run the solution after the mixture had been filtered. The extracted extract was dried and kept at -22°C and preserved for later use.

Phytochemical Estimation

The total phenolic contents in the extracts of *T. natans* were assessed using a standard curve made of Gallic acid. A biochemistry analyzer (Biolab-310®, URIT Medical Electronic Co., Ltd., China) was used to measure the absorbance at 760 nm wavelength. The extract's total flavonoid contents were evaluated using a standard curve of

quercetin, by the method used in previous study by Kumar (Sandhya et al., 2018). Absorbance was taken at 540nm using a chemistry analyzer (Biolab-310®, URIT Medical Electronic Co., Ltd., China). Sample solution (5µl) of 5 different concentrations 5, 2.5, 1.25, 0.62 and 3.12 mg/ml made in methanol was mixed with 585µl solution and solution of 0.2 percent 1,1-Diphenyl-2-picrylhydrazyl (DPPH) in methanol was left for 20 minutes at room temperature. At 515nm, the absorbance was taken using a Biolab-310", (URIT Medical Electronic Co., Ltd., China) chemistry analyzer. A graph constructed between percent inhibition and amount of the sample was used to estimate the amount of plant extract with 50% scavenging ability (Sethi et al., 2020).

High Performance Liquid Chromatography (HPLC)

The HPLC system (HP 1050 gradients) was equipped with a detector (SOD-10AV) and 10µl of plant extract (0.1 g/ml in methanol) in order to estimate the phenolic profile by HPLC. The Shim-Pack CLC-ODS C-18 column (5cm × 4.5mm, 5µm) Shimadzu Japan® was utilized as the stationary phase. As the mobile phase, distilled water and glacial acetic acid were combined at a v/v ratio of 24:0.4:320:56. At room temperature, different phenolic contents were determined using a linear gradient that lasted 10 minutes (Hussain et al., 2019).

Physical Parameters

The body weight of each rat was measured once in a week during the experimental period, and daily food and water consumption were recorded. And conscious rats were gently restrained in a plastic chamber while tail-cuff plethysmography were performed to monitor their systolic blood pressure (SBP). The session average from three readings was then noted (Otero-Losada et al., 2016).

Electrocardiogram (ECG)

ECG was performed on each group of rats using a Power lab (15T; Model ML 4818) to assess the effects of the medication on heart rate as well as changes in heart rate brought on by fructose-induced hypertension (Dianat et al., 2014).

Measurement of oxidative stress markers

Estimation of serum oxidative stress markers was done using the Total oxidant status assay kit manufactured by PhysioTech, catalogue no# 002 and absorbance of serum sample were taken at 546 nm and Total antioxidant Assay kit, catalogue no# 001 following (Saleem et al., 2025) and absorbance were taken at 630 nm by biochemistry analyzer (BIOLAB-310). For the determination of malondialdehyde (MDA), serum sample absorbance were taken spectrophotometrically at 532 nm using the kit method of

PhysioTech catalogue no# 003 following (Saleem et al., 2025).

Serum renal function test

The blood level of uric acid was measured using the uric acid-Liquizyme, Uricase-PAP (single Reagent, Spectrum, catalogue # 323000) reagent kit strategy. To measure serum creatinine, the (Creatinine-Jaffe, Spectrum, catalogue # 234001) reagent kit was utilized.

Liver function test

Using Jeddah's Crescent® Diagnostic kit (catalog # 15204C), the serum AST concentration was measured. The serum ALT level was measured using a kit (ALT (GPT) SR, by Bio-active, catalog # 10498 99 93 183).

Lipid profile

To quantify total cholesterol (TC), the Cholesterol-Liquizyme (by spectrum catalog # 230004) reagent kit method was used. Using the (TRIGLYCERIODS SR* Diagnostic kit, catalog # 10498 99 93 204) Kit technique, triglycerides were assessed. HDL was determined using the HDL-cholesterol phosphotungstic precipitation technique (catalog #CS009 1100) kit method from Bio Research. LDL was measured by the given formula:

$$\text{LDL} = \text{Total Cholesterol} - \text{Triglycerides}/5 - (\text{HDL})$$

Histopathology

Following a 24-hour fixation period in 10% paraformaldehyde, tissue from the heart, kidney, and aorta was dehydrated using varying ethanol concentrations. After being encased in paraffin wax, the tissues were cut using a microtome into slices that were 2 to 4 µm thick. Eosin and haematoxylin stains will thereafter be applied to the deparaffinized sections, and they will be examined under a light microscope (Huang et al., 2015). The degree of heart, aorta and kidney tissue damage were determined by a senior pathologist via a point scoring method using an ordinal scoring scale: point 0, no injury evidence; point 1, minimal injury in heart, aorta and kidney tissues that was slight decrease of muscle striation in heart tissues, reduced tunica media thickness in aortic tissues and reduced interstitial vacuolation in glomeruli in kidney tissues; point 2 modest to severe injury with muscle distraction with dispersed inflammatory cells in heart tissues, increased tunica media thickness in aortic tissues and hypercellular glomeruli, and destroyed tubules in kidney tissues.

Gene expression study

Triazole (Therma Fisher Scientific) was used to preserve an inch of heart muscle tissue at -40°C until it is processed further. The amount of extracted RNA was measured using

Nanodrop (Thermo Scientific). Following the manufacturer's instructions, equal concentrations of RNA from each sample was used in the RevertAid cDNA synthesis kit (Thermo Fisher Scientific) to perform the cDNA synthesis. qRT-PCR was used to express the genes for ACE, iNOS, eNOS, COX2 and NFkB (Biorad; BM10-QPCR96 RT 2).

Statistical analysis

Two-way ANOVA were used to examine the dataset in order to determine if the treated and untreated groups were statistically significant. Post-significance tests were conducted using the Tukey test (GraphPad Prism 9.1.2; California) software, and significant differences were identified by reaching significance threshold ($P \leq 0.05$).

Results

Qualitative analysis

Qualitative analysis of *Trapa natans*, is expressed in (Table.1) showing the phytochemicals presence in the Methanolic extract of *T. natans* (MeTN) including phenols, flavonoids, alkaloids, reducing sugar, anthraquinones, terpenoids, tannins saponins and steroids in extract.

Phytochemical analysis

Methanolic extract of *T. natans* peel (MeTN) results was found to contain (4.2 ± 0.5 mg QE/g) of flavonoid contents and the phenolic contents was (59.8 ± 1.9 mg GAE/g) dry weight of plant.

Determination of radical scavenging activity by DPPH

Methanolic extract of *Trapa natans* peel (MeTN) showed and IC₅₀ value and DPPH radical scare activity in dose dependent manner as shown in (Figure 1).

experimental trial. The body weight of standard (STD) group showed significant ($P \leq 0.05$) reduction except for healthy rats (NC). However, the body weight showed significant ($P \leq 0.05$) reduction in treatment groups (TR; 149 ± 0.58 g) as compared to positive control group (PC) at 8th week, as shown in (Figure 3).

Blood Pressure (BP)

The overall systolic and diastolic blood pressure (Mean $\pm$ SEM) in (PC; hypertensive rats) showed significant ($P \leq 0.05$) elevation throughout the experimental trial. The systolic blood pressure, and diastolic blood pressure of standard group (STD) was significantly ($P \leq 0.05$) decreased except negative control groups. While the treatment groups of both systolic blood pressure (130.67 ± 1.20 mmHg) and the diastolic blood pressure (84 ± 1.53 mmHg) were significantly decreased as compared to positive control (PC) group on 14th day as shown in (Figure 4).

Oxidative Stress Parameters

The total oxidant status (Mean $\pm$ SEM) and serum malondialdehyde level showed significant ($P \leq 0.05$) increase while the serum total antioxidant capacity showed significant ($P \leq 0.05$) reduction (PC; hypertensive rats) throughout the experimental trial. The total oxidant status and serum malondialdehyde level showed significant ($P \leq 0.05$) elevation, while the total antioxidant capacity was significantly ($P \leq 0.05$) increased in standard (STD) groups. After the treatment, the serum TOS and serum MDA levels of treatment groups significantly ($P \leq 0.05$) decreased and serum TAC level showed significant ($P \leq 0.05$) elevation as compared to the positive control (PC) groups at on 14th day, as shown in (Figure 5).

Renal Function Test

Table 1. *In vitro* quantitative phytochemical analysis of *Trapa natans*

Phytochemicals	Test	MeTN
Carbohydrate	Fehling's test	+
	Bendict's test	+
Proteins	Xanthopoietic	++
Alkaloids	Hager's reagent	+++
Flavonoids	NaOH	+++
Phenols & tannins	Lead acetate test	+++
Steroids & terpenoids	Chloroform	++

(+++); strongly positive, (++); moderately positive, (+); weakly positive, (-); not detected.

Body weight

The mean body weight (Mean $\pm$ SEM) in (PC; hypertensive rats) showed significant ($P \leq 0.05$) elevation throughout the

The overall serum uric acid level and serum creatinine level (Mean $\pm$ SEM) in (PC; hypertensive rats) showed significant

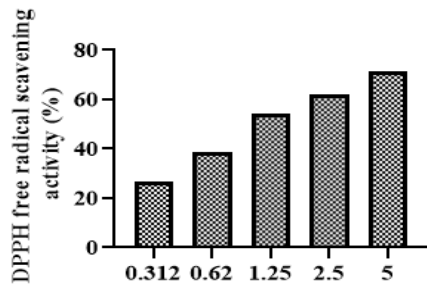


Figure 1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of 5 different concentrations of Methanolic extract of *Trapa natans* peel.

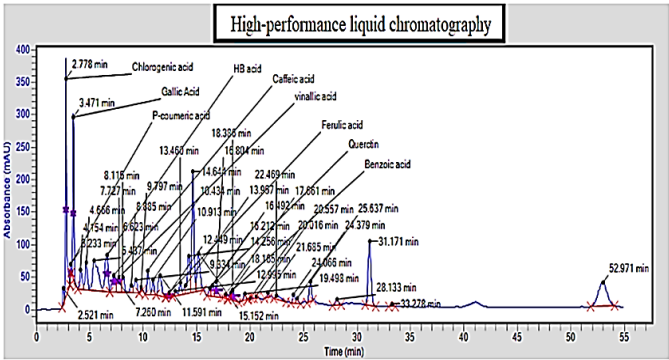


Figure 2. High-performance liquid chromatography of the methanolic extract of *Trapa natans* peel.

Table 2. High-performance liquid chromatography of *Trapa natan*

Component Name	RT(min)	Area	K factor*Area	Amount (mg/kg dry extract)
Chlorogenic acid	2.778	2176058	282.88754	0.2828
P-coumaric acid	3.233	59567.1	19.8358443	0.019836
Gallic acid	3.471	1911255	168.1904136	0.16819
HB acid	6.623	1157452	185.19224	0.185192
Caffeic acid	7.26	582808.5	343.857015	0.343857
Vinallic acid	7.727	514750.4	399.9610608	0.399961
Ferulic acid	12.449	131511.2	100.8690904	0.100869
Quercetin	16.804	397553.1	276.6969576	0.276697
Benzoic acid	18.386	260304.8	8.9805156	0.008981

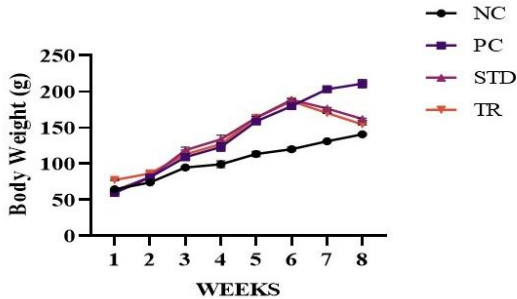


Figure 3. Body weight (gram; Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard (STD; Amlodipine 5mg/kgbw), and TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose induced hypertension.

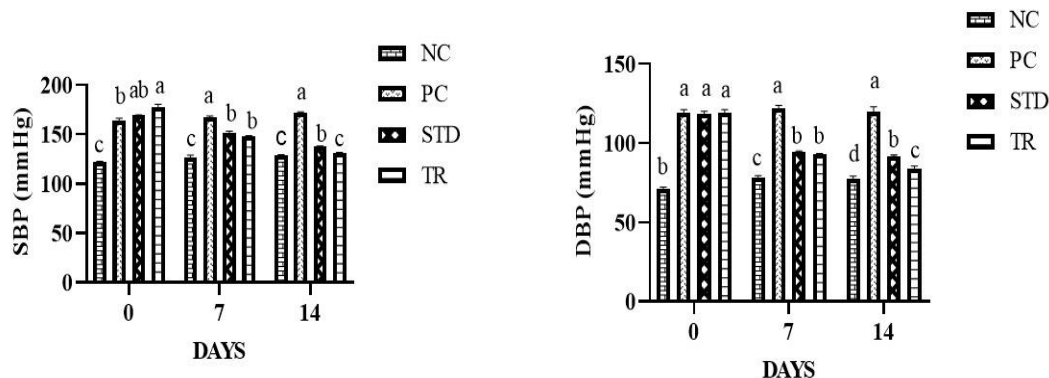


Figure 4. Systolic and diastolic blood pressure (mmHg; Mean $\pm$ SEM) in Negative Control (NC), Positive Control (PC), Standard (STD; Amlodipine 5mg/kgbw), and TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-c}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

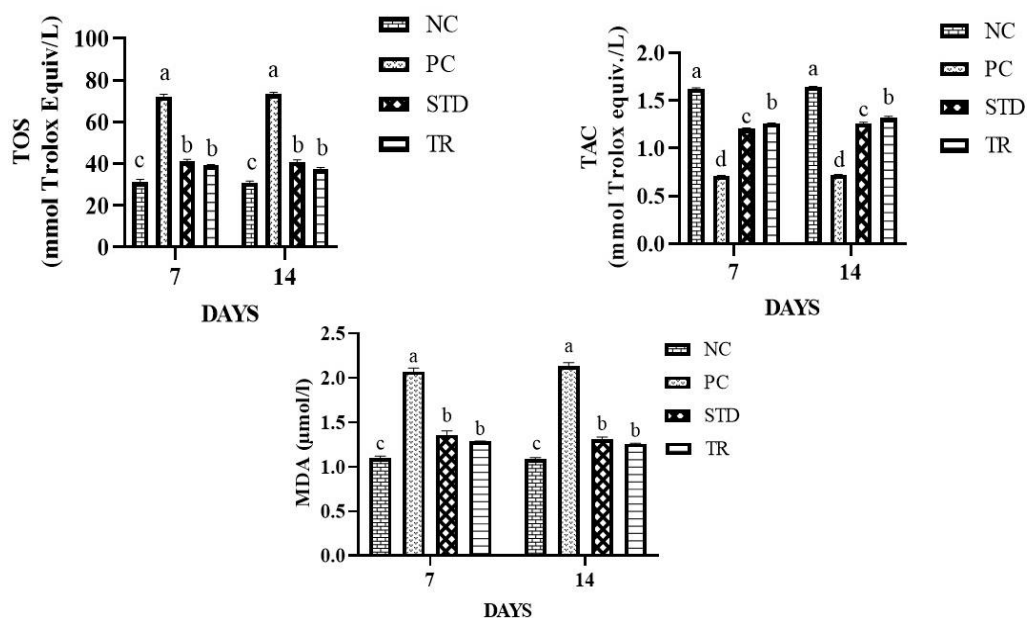


Figure 5. Total Oxidant Status, total antioxidant capacity and MDA (Mean $\pm$ SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose induced hypertension. (^{a-c}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

($P \leq 0.05$) elevation throughout the experimental trial. The, serum uric acid level and serum creatinine level of standard group (STD) was decreased significantly ($P \leq 0.05$) except negative control groups. While the treatment groups of both serum uric acid level (2.45 ± 0.06 mg/dl) and the serum creatinine level (0.83 ± 0.015 mg/dl) were significantly

decreased as compared to positive control (PC) group on 14th day as shown in (Figure 6).

Serum Liver Function Test

The overall serum aspartate aminotransferase and serum

alanine Transaminase level (Mean $\pm$ SEM) in (PC; hypertensive rats) showed significant ($P\leq 0.05$) elevation throughout the experimental trial. The, serum AST and serum ALT level of standard group (STD) was decreased significantly ($P\leq 0.05$) except negative control groups. While the treatment groups of both serum aspartate aminotransferase level (40.84 ± 0.88 U/L) and the serum alanine Transaminase level (43.07 ± 0.88 U/L) were significantly decreased as compared to positive control (PC) group at on 14th day showed in (Figure 7).

Serum Electrolyte Concentration

The overall serum sodium concentration (Mean $\pm$ SEM) showed a significant ($P\leq 0.05$) reduction, and serum potassium concentration showed a significant ($P\leq 0.05$) reduction in (PC; hypertensive rats) throughout the experimental trial. The decreased serum sodium concentration was evidently significant ($P\leq 0.05$) and serum potassium concentration was significantly ($P\leq 0.05$) increased in standard group (STD) except negative control groups. While the treatment groups of serum sodium concentration showed significant ($P\leq 0.05$) decrease (104 ± 1.15 mmol/L) and serum potassium concentration showed significant increase (5.5 ± 0.057 mmol/L) as compared to positive control (PC) group at on 14th day as shown in (Figure 8).

Lipid Profile

The total cholesterol, total triglyceride and LDL level showed significant ($P\leq 0.05$) elevation while, the HDL level was significantly ($P\leq 0.05$) reduced in (PC; hypertensive rats) throughout the experimental trial. However, the TC, TG and LDL level showed significant ($P\leq 0.05$) reduction in standard group (STD) and the HDL showed significant ($P\leq 0.05$) reduction except normal control (NC). After the end of the trial the total cholesterol (82.8 ± 1.36 mg/dl), total triglyceride (114 ± 1.26 mg/dl) and low-density lipoproteins (41 ± 0.57 mg/dl) level were decreased significantly ($P\leq 0.05$) while the high-density lipoproteins (61.7 ± 0.8 mg/dl) were increased significantly ($P\leq 0.05$) as compared to positive control (PC) as shown in (Figure 9).

Gene expression analysis

The Expression level of ACE, eNOS, iNOS, COX-2, NFK- β (Mean $\pm$ SEM) was measured between different groups as shown in (Figure 9). The expression level of ACE, eNOS, iNOS, COX-2, NFK- β showed significant ($P\leq 0.05$) increased in (PC; hypertensive rats) throughout the experimental trial. However, the expression level of ACE, eNOS, iNOS, COX-2, NFK- β was decreased significantly ($P\leq 0.05$) in standard (STD) group except normal control (NC) group. After the end of the trial the expression level of ACE (2.06 ± 0.04), eNOS (1.97 ± 0.02), iNOS (2.22 ± 0.02),

COX-2 (0.95 ± 0.02), NFK- β (1.40 ± 0.02) showed significant ($P\leq 0.05$) reduction in treatment group as compared to positive control group showed in (Figure 10).

Heart rate

The overall heart rate was increased significantly ($P\leq 0.05$) in (PC; hypertensive rats) throughout the experimental trial. However, the heart rate was decreased significantly ($P\leq 0.05$) in the standard group (STD) except normal control (NC). After the end of the trial the heart rate of the treatment group (TR; 166.7 BPM) was decreased significantly ($P\leq 0.05$) in comparison to positive control as showed in (Figure 11).

Histopathology

Histopathology of heart tissue, aortic tissue and kidney tissues samples of different groups is shown in (Figure 12). In the histological examination of the heart tissues, the negative control (NC) group showed normal myofibrillar structure, positive control (PC) group (Fructose fed hypertensive rats) showed muscle distraction with dispersed inflammatory cells and some cellular infiltration to myositis indicating grade 2 injury, standard (Amlodipine treated) group (STD) revealed a slight decrease of heart muscle striations as well as sporadic muscular distraction, treatment (TR) group (*T. natans* extract 200 mg/kg) demonstrated that the histological appearance of the congested myocardium had practically returned to near-to-normal histology as showed in (Figure 12a). In the histology of aortic tissues, the tunica intima (TI) faces the lumen in the negative control (NC) group, the tunica medium (TM) is made up of smooth muscle and elastic fibers, and the top layer, the tunica adventitia (TA), is composed of loose connective tissue. The (PC) positive control group (fructose hypertensive) had pathological alterations revealing grade 2 injury, such as increased tunica medium thickness, medial inflammatory cell infiltration, and lumen bulging. Standard (STD) control (Amlodipine treated group) showed a reduced TM thickness. Methanolic extract of *T. natans* (200mg/kg) shown a reduction in wall thickness, normal endothelium lining, and muscle structure as showed in (Figure 12b). Histology of kidney tissues revealed that, the negative control group (NC), normal glomeruli and tubule histological structure was observed. A range of pathogenic characteristics were present in the fructose feeding model positive control group (PC), including interstitial haemorrhage, hypercellular glomeruli, and destroyed tubules associated with swollen epithelial cells indicating grade 2 injury. Histopathological damage brought on by fructose ingestion was observed after treatment with Methanolic extract of *Trapa natans* (200mg/kg). A practically normal histological renal pattern was seen in the Methanolic extract of *Trapa natans* (200mg/kg), and the

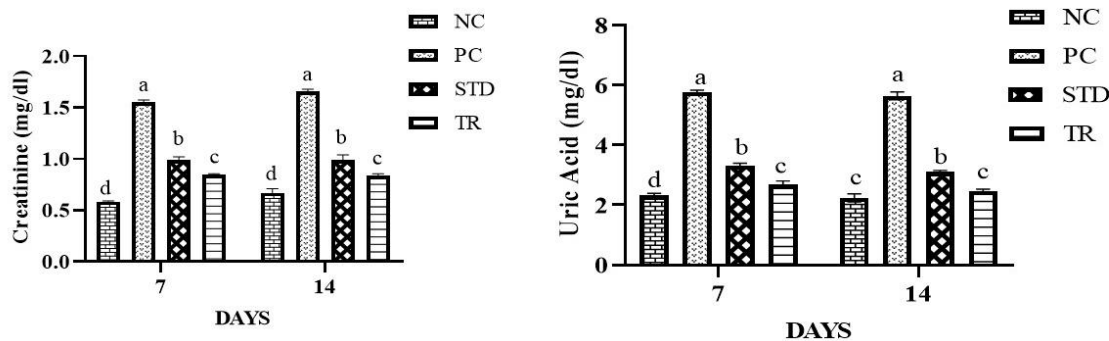


Figure 6. Uric acid and creatinine (Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-d}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

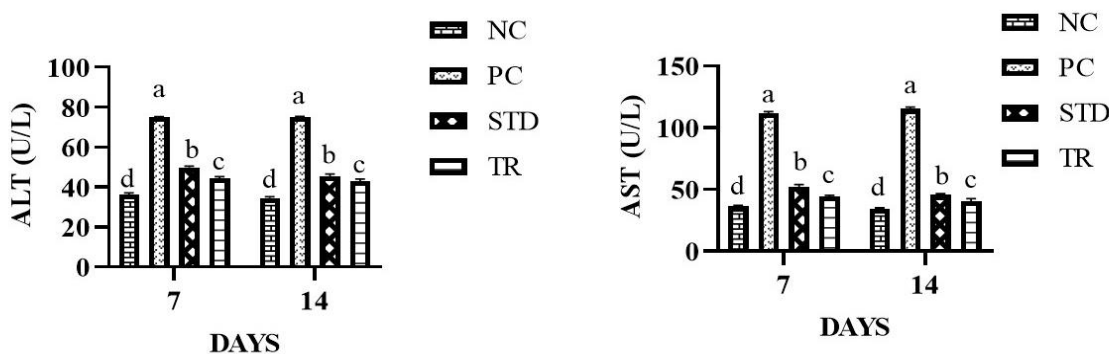


Figure 7. Aspartate aminotransferase, Alanine Transaminase (Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-d}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

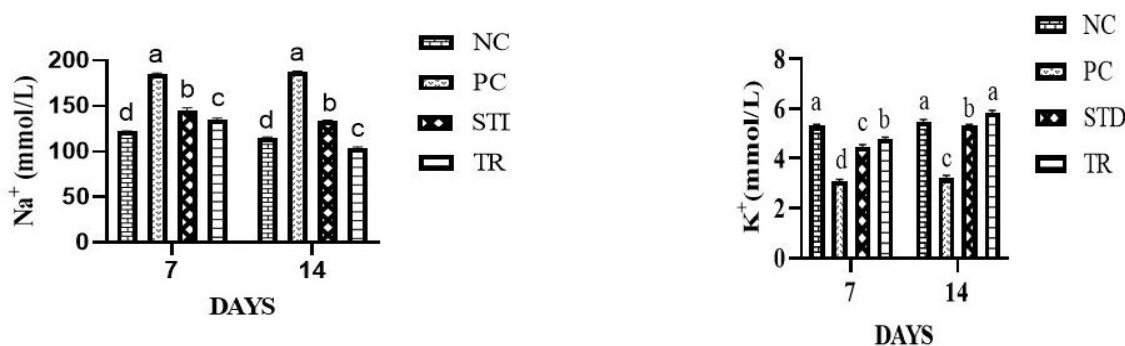


Figure 8. Serum sodium and potassium concentration (Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (Methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-c}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

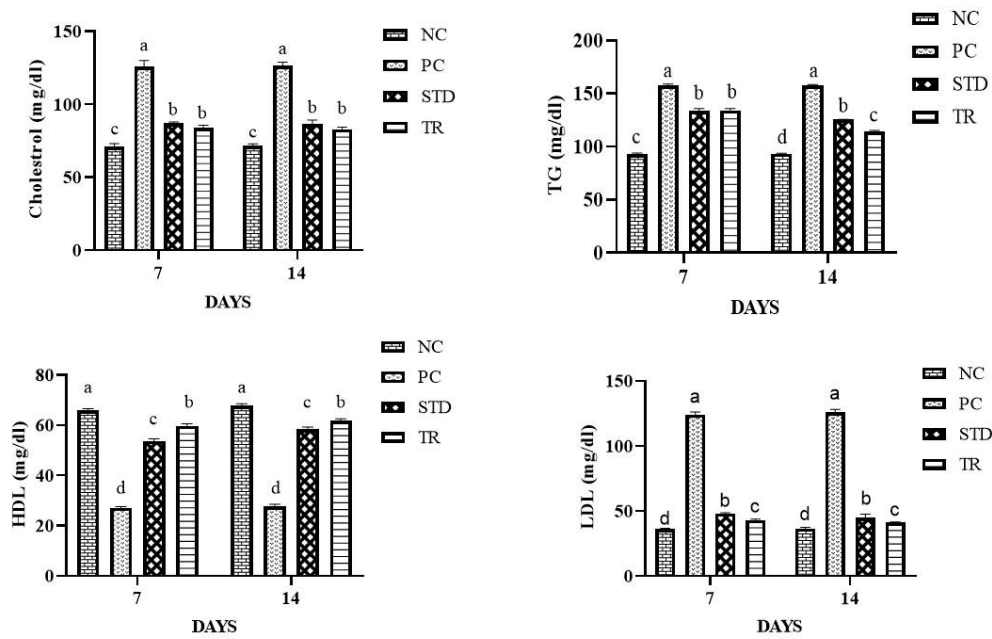


Figure 9. Cholesterol, TG, HDL, and LDL (Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-c}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

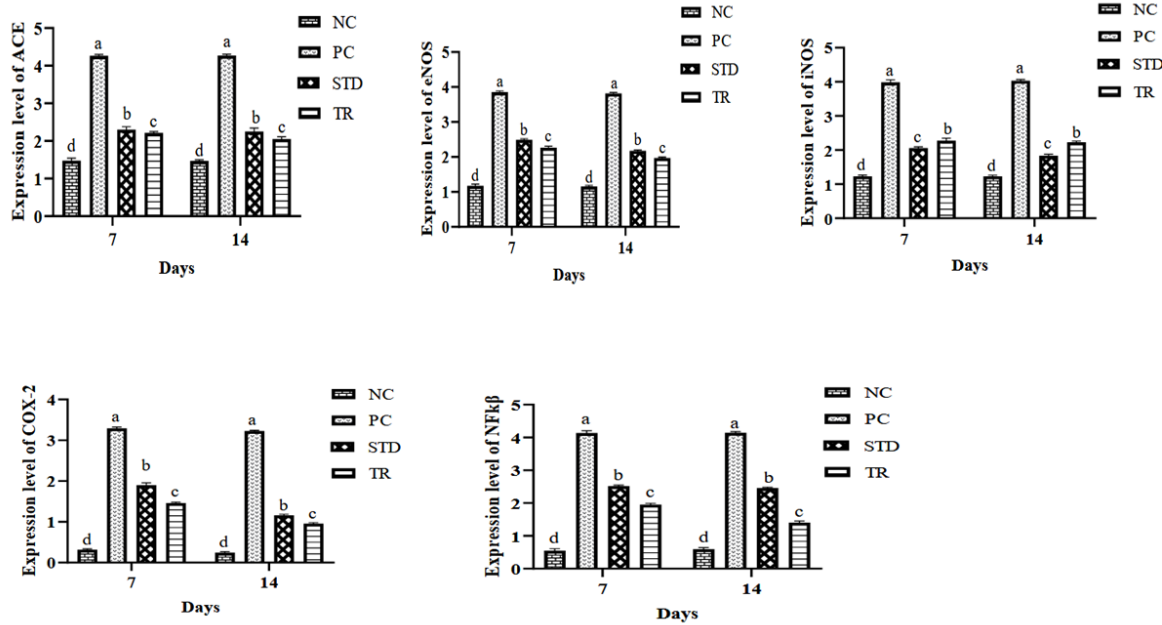


Figure 10. Expression level of ACE, eNOS, iNOS, COX-2, NFK-β (Mean±SEM) in Negative Control (NC), Positive Control (PC), Standard Control (STD; Amlodipine 5mg/kgbw), TR (methanolic extract of *Trapa natans* peel 200mg/kgbw) at different days, in fructose-induced hypertension. (^{a-c}) There is a substantial difference ($P \leq 0.05$) between the letters on the bars.

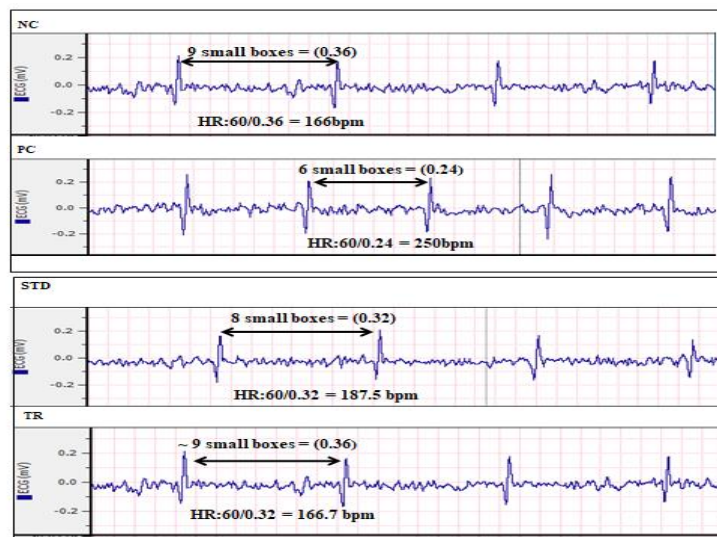


Figure 11. Charts for (ECG) in Negative Control group (NC), Positive Control group (PC) (fructose induced hypertensive rat), Standard group (STD) Amlodipine treated, and TR (methanolic extract of *Trapa natans* 200mg/kg).

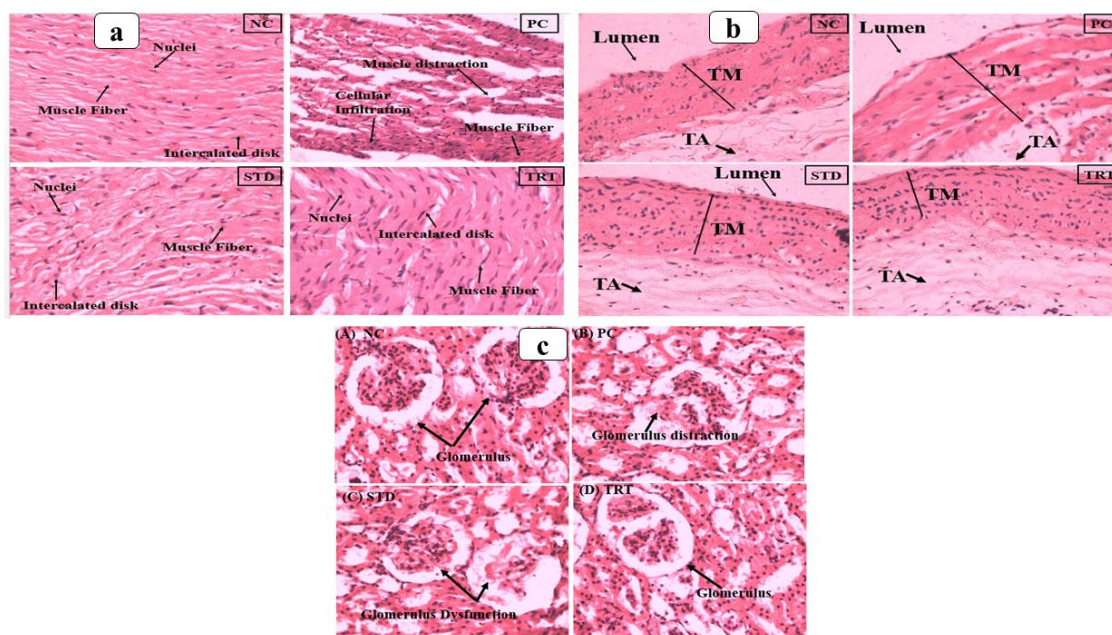


Figure 12. Photomicrograph (H&E staining; 40X) demonstrating the histology of rat heart tissue (a), aorta tissue (b), kidney tissue (c) at 14th day, in Negative Control (NC), Positive Control (PC), Standard Control (STD) and Treatment Group (TR).

interstitial vacuolation in the glomeruli was reduced by the standard treatment (STD), Amlodipine treatment group as shown in (Figure 12c).

Discussion

Hypertension is a worldwide disorder, different studies found that 34% of individuals worldwide suffer from hypertension, 58.7% were aware of their condition, and 54.7% were taking

medication to treat it (Beaney et al., 2020). The general population's life expectancy is greatly shortened by hypertension, which increases the risk of various disorders such as ischemic heart disease, chronic renal disease, aortic dissection, and stroke (Kleinrok et al., 2017).

Numerous plants in traditional medicines have proven to be used to treat various diseases, and the use of phytochemicals has been enhanced drastically for various illnesses in light of

their low cost, and trouble-free availability and it is also believed that natural cures have a less destructive impact when contrasted with synthetic medicines (Adib-Hajbaghery & Rafiee, 2018). *T. natans* peel are proved to be a nutraceutical plan that has massive therapeutic and dietary benefits and advantages (Alam et al., 2021).

In our study, rat model of hypertension was created by feeding 10% fructose in drinking water for 6 weeks, along with a chow pellet diet (Mondal et al., 2019). After experimental induction of hypertension, body weight was increased in hypertensive fructose-fed rats as compared to healthy rats. Fructose rich diet also exhibits metabolic syndrome causing insulin resistance. Due to an increase in hepatic triglyceride production, it also results in hypertriglyceridemia and weight gain (Chaudhry et al., 2021). In *T. natans* treated group, body weight was significantly declined as contrasted with the positive control group as polymeric proanthocyanidins are present in *Trapa natans* peel, which have great anti-obesity effect (Kimura et al., 2011).

Blood pressure in hypertensive rats was increased as compared to healthy rats throughout the experiment. Because, fructose can increase blood pressure through a number of different processes, including salt overload, vasoconstriction, and sympathetic activation (Klein & Kiat, 2015). In treatment group (methanolic extract of *T. natans* peel), blood pressure was decreased compared to positive control group. According to one study, those with high blood pressure who took more potassium had their systolic and diastolic blood pressure drop by 3.49 mmHg and 1.96 mmHg, respectively. These individuals also had a lower chance of getting heart attacks (Rani et al., 2016). *T. natans* peel are therefore those types of fruit that are a rich source of potassium and help to lower the blood pressure and stroke risk (Zhang et al., 2022). In the current study, fructose induced hypertensive rats showed higher level of serum total oxidant status and malonaldehyde and decreased level of TAC as compared to the normal control group. However, increase in total antioxidant capacity was due to phenolic compounds such as flavonoids possess antioxidant property. The methanolic extract of *T. natans* peel showed higher antioxidant capacity and radical scavenging activity than vitamin C (Kim et al., 2014).

Rats given with high-fructose diet showed rise in alanine aminotransferase and aspartate aminotransferase enzyme levels (Chung et al., 2014). The fructose group in this experiment had the highest levels of the liver enzymes AST, ALT, and low-density lipoprotein activity compared to the healthy rats and the activity of these enzymes was reduced in rats treated with methanolic extract of *T. natans* peel. According to a study, the CCl₄-induced increase in the cell's alanine and aspartate aminotransferase activities were reduced by *T. natans* peel extract as compared to Silymarin,

the extract showed even more potential for hepatoprotection (Wang et al., 2011).

In the current study, dyslipidaemia was evident in the fructose-fed rat group, with lower high-density lipoproteins and increased in serum triglyceride level, low-density proteins, and total cholesterol levels compared to the negative control group (Geidl-Flueck & Gerber, 2017). However, in *T. natans* treated group, triglycerides, low-density lipoproteins and total cholesterol was decreased and serum high-density lipoproteins level was elevated as compared to hypertensive rats.

Serum sodium level was high and potassium level was decreased in fructose feeding to hypertensive rats as compared to healthy rat group. Intrarenal RAAS is also elevated during extended durations of fructose and high salt consumption, which could promote tubular Na⁺ reabsorption even more. Afferent nerve inputs, reactive oxygen species, and elevated metabolic hormones like leptin all work together centrally to activate sympathetic outputs and raise blood pressure (Komnenov et al., 2019). However, because of methanolic extract of peel of *T. natans*, that are heavy in potassium, with one cup containing 362.1 mg, the potassium level rose in the *T. natans* treated group. This electrolyte is necessary for health of brain and function of muscles. Controlling salt influences blood pressure and water retention (Singh et al., 2017).

The electrocardiograms (ECG) from the PC group in our study, as demonstrated in (Figure 11), showed shorter the R-R interval, shortened QRS complex, and hidden P-wave which eventually showed a state called supraventricular arrhythmia, which is led to asymmetrical heartbeat that starts over the ventricles and makes the heart beat too rapidly and erratically. When compared to the hypertensive rats, the condition was significantly improved in rats treated with the methanolic extract of *T. natans* peel and standard treated group (treated with amlodipine) groups, where the R-R interval was normal and the heart rate was lower.

The results of (H&E) staining used to examine the heart tissue are shown in (Figure 12a), a typical myofibrillar structure with striations, intact myocytes, cell membranes, and the presence of branching and continuous myofibrils was seen in negative control group. Rats treated with fructose in positive control group developed a number of pathological characteristics in their cardiac tissues, including muscle separation and cells that seemed to be vacuolated. The phytotherapy of methanolic extract of *T. natans* peel reduced myocardial infarction as compared to hypertensive rats, and myocardium returned to almost normal histological appearance. The rats of amlodipine treated standard group showed myocardial fibers which were tightly packed, demonstrating an incomplete lack of heart muscle striations and atypical muscle separation. The histological examining of aortic tissue of rats, H.E. staining data are shown in (Figure

12b) showed that control group exhibited intermingled smooth muscle cells and wavy elastic collagen fibers in the tunica media. The endothelium bulged and tore in the fructose feeding positive control group due to an unorganized buildup of fibers and layers of smooth muscle cells in the subintima have increased. However, in the standard group (Amlodipine treated), tunica media of aorta showed smooth muscle cells and wavy elastic collagen fibers and treatment group (methanolic extract of *T. natans* peel) showed lower fibrosis and the aortic wall with more collagen deposition. While in histology of kidney tissues, the negative control group, normal glomeruli and tubule histological structure was observed. A range of pathogenic characteristics were present in the fructose feeding model group (positive control group), including interstitial haemorrhage, hypercellular glomeruli, and destroyed tubules associated with swollen epithelial cells. Histopathological damage brought on by fructose ingestion was improved after treated with methanolic extract of *T. natans* peel. A practically normal histological renal pattern was seen in the methanolic extract of *T. natans* peel therapy, and the interstitial vacuolation in the glomeruli was reduced by the standard group (Amlodipine treated group) as shown in (Figure 12c).

The mRNA expression level of several genes implicated in a hypertension pathology was examined and observed in the current study by the use of qRT-PCR (qualitative real-time PCR) based analysis. Endothelial cells produce and release contracting and relaxing substances, which are essential for controlling vascular tone. Consuming fructose can cause endothelial dysfunction through a number of different mechanisms, which can ultimately result in hypertension. C-reactive protein, cyclooxygenase-2 (COX2), Ang II, advanced glycation end products (AGEs), eNOS, NO, and reactive oxygen species. Endothelial nitric oxide synthase (eNOS) produces nitric oxide, a potent vasodilator that lowers blood pressure. By decreasing the activity and expression of eNOS, fructose feeding has been demonstrated to reduce nitric oxide generation. It has been demonstrated that *T. natans*' flavonoids and phenolic compounds lower ACE and cyclooxygenase-2 (COX-2) expressions. The main aim of this study was, the systolic blood pressure in the hypertensive rat model should decrease when the *T. natans* extract was administered. Strong evidence at the molecular level supports this robust physiological result. In particular, we discovered that the expression of the ACE gene was downregulated in heart tissues while eNOS were significantly upregulated in the vascular tissue (Figure 10). This dual modulation points to a very efficient, well-coordinated blood pressure regulation system. Angiotensin II, a strong vasoconstrictor, is produced less often when ACE, the main enzyme in the Renin-Angiotensin System (RAS), is suppressed. At the same time, increased eNOS activity raises nitric oxide's (NO) bioavailability, which encourages

vasodilation. Thus, our findings imply that *T. natans* inhibits vasoconstrictor pathways while simultaneously stimulating vasodilatory pathways to provide its antihypertensive action. Although earlier studies have emphasized *T. natans*'s overall antioxidant and hepatoprotective qualities (Asha et al., 2024) our results offer the first mechanistic connection between the plant's phytochemical makeup and its direct influence on important cardiovascular-regulating genes. The main innovation of this work is the shift from broad pharmacological observation to precise molecular targeting, which supports the use of *T. natans* as an effective treatment for hypertension. Although, current study needs further evaluation of cardioprotective benefits of *T. Natans* in clinical trial along with extract evaluation for isolation of compounds as new entities.

Conclusion

The current study revealed the therapeutic and protective efficacy of methanolic extract of *Trapa natans* peel (water chestnut) in fructose induced hypertensive rat model through normalizing arterial blood pressure, heart rate, oxidative stress, electrolytes balance, lipid profile and safety through LFTs and RFTs. However, the histological and ECG changes confirm the antihypertensive potential of *Trapa natans* peel. Our research suggests that water chestnut may be helpful in the medical nutrition therapy to treat hypertension. To further clarify its mode of action, additional research studies are needed.

Author Contributions

Noreen Kousar: performed experiment, Iman Aslam: Data collection, Ghazala Nayab, Sammar Zaitoon, Formal analysis. Tahseen Akhtar, Kashaf Masood: Writing original draft, Review & editing. Sana Saleem, Janita Ashiq and Saira Muqadas: Methodology. Haseeb Anwar, Imran Mukhtar: Conceptualization, Project administration, Supervision

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Conflicts of Interest

The authors declare no conflict of interest.

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