Research article

To study cardioprotective effect of ethanolic extract of *Withania coagulance* dunal fruit on fructose induced metabolic syndrome in experimental animals.

Mohini B. Jadhav[1], Deepti D. Bandawane*, Trupti B. Gadhave[2], Pratiksha Hajare[3].

Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44, Maharashtra, India *

ABSTRACT

Introduction: Metabolic syndrome (MetS), characterised by insulin resistance, hypertension, and dyslipidemia, poses a significant risk for cardiovascular disease.10% (w/v) fructose solution to drink ad libitum are known to exacerbate metabolic syndrome. *Withania coagulans* (WC) is a medicinal plant traditionally used for its anti-inflammatory and antioxidant properties. This study aims to investigate the cardioprotective effects of *Withania coagulans* fruit extract in a high fructose induced metabolic syndrome model.

Methods: Male Wistar rats were divided into five groups: normal, diseased 10% (w/v) fructose water ad libitum for 9 weeks, 10 % (w/v) fructose + metformin (300mg/kg), 10 % (w/v) fructose +EEWC at dose of (200 mg/kg), 10 % (w/v) fructose +EEWC at dose of (400 mg/kg). The diseased groups received a 10 & (w/v) fructose solution to drink ad libitum for 9 weeks, while the EEWC groups were additionally administered *Withania coagulans* fruit extract at low (200 mg/kg) or high (400 mg/kg) doses. Cardiovascular parameters, lipid profiles, glucose levels, and inflammatory biomarkers were assessed.

Results: The 10 % (w/v) fructose solution to drink ad libitum significantly increased body weight, serum glucose, total cholesterol, triglycerides, and inflammatory biomarkers while decreasing HDL cholesterol. Administration of *Withania coagulans* fruit extract markedly improved these parameters. The 10 % (w/v) fructose +EEWC at dose of (400 mg/kg) group showed the most significant reduction in serum glucose, cholesterol levels, and inflammatory biomarkers, and an improvement in HDL cholesterol. Histological examination of heart tissues indicated a reduction in inflammatory cell infiltration and fibrosis in the *Withania coagulans* treated groups.

Key words: Metabolic syndrome, Homocysteine, Castelli risk index I & II, Insulin resistance, etc.

Introduction

The term "metabolic syndrome" (MetS) refers to a cluster of metabolic risk factors that includes atherogenic dyslipidemia, insulin resistance, hypertension, and central obesity. MetS is highly correlated with an increased risk of cardiovascular disease and type 2 diabetes. Central obesity, hypertension, insulin resistance, and atherogenic dyslipidemia are the factors that control MetS [1]. The etiology of Metabolic Syndrome is also influenced by genetic and lifestyle variables [2]. Because obesity rates are so high globally, Meta-analysis has received a lot of attention lately [1]. Patient's diagnoses are crucial in order to modify lifestyle choices and risk factors and thereby change the course of the illness. For people diagnosed with metabolic syndrome, losing weight, eating a balanced diet, and exercising are the most crucial lifestyle modifications. MetS is an issue that exists everywhere. A higher chance of having metabolic syndrome is linked to abdominal fat. Different nations and locations have different rates of the condition depending on a variety of criteria, such as gender, age, ethnicity, and the diagnostic definition utilized. Growing older increases one's chance of developing MetS. Less than 10 % of young adults in their twenties and 40 % of elderly in their sixties are affected by MetS. Over 45 million adults in the US are affected by MetS, accounting for more than fifth of the population [3].

Research over the past several years has demonstrated that eating fructose causes oxidative damage to promote metabolic disruption. Furthermore, an increasing fructose intake may be a significant factor in the metabolic syndrome, according to a number of studies. Additionally, eating a diet high in fructose causes a well-known metabolic syndrome, which is characterised by a decreases in HDL cholesterol and an increase in hypertension, hyperinsulinemia, insulin resistance, and hypertriacylglycerolemia. Furthermore, studies on animals fed a high-fructose diet have demonstrated modified lipid metabolism as a result of hepatic stress brought on by the load of fructose metabolism [4]. MetS components have been demonstrated to be improved by a few medicinal plants and dietary components. Crucially, a number of medicinal plants can lower blood pressure, blood fat percentage, triglycerides, plasma cholesterol, insulin sensitivity, and low-density lipoprotein cholesterol [5].

Any plant that has a chemical with therapeutic qualities or that has a positive pharmacological effect on people or animals can be considered medicinal. Plants, herbs, food plants, spices, and microscopic plants are all considered medicinal plants [6]. Due to the low side effects of medicinal herbs, there is currently a growing interest in these plants and their extracts.

The plant *Withania coagulans* a well-known medicinal plant in indigenous medicine, dunal is a member of the solanaceae family and is mostly found in the eastern Mediterranean area, which stretches to South Asia. It may be found throughout most of India [7]. Just two of the twenty-three species of the genus *Withania- W. coagulans* and *W. Somnifera-* have been found to be economically significant [8]. Because the berries of this plant contain an enzyme that causes milk to clot, it is popularly referred to as an Indian cheesemaker [9]. The fruit, leaves, and roots are all medicinally beneficial. Berries are mostly composed of alkaloids, amino acids, esterases, and essential oils [10]. Steroids derivative chemicals called "withanolides" are thought to be responsible for the plant's therapeutic qualities. Within the whole plant, there are many withanolides, including coagulin F, caoagulanolide, withacoagulin, and coagulin G [9]. Ripe fruit from the plant is pleasant and has been used as a sedative for asthma, dyspepsia, and wound healing. Dry fruit is also a common traditional diabetic therapy in several nations [11],

and as an antibacterial [12], antimicrobial [13], hepatoprotective [14], hypolipidemic [15], antioxidant [16], anti-tumor [17], antidepressant [18], immunosuppressive [19], and anti-inflammatory agent [20]. While flowe buds demonstrated an anthelmintic effect, seeds are beneficial for lowering inflammation, acting as a diuretic, and treating ophthalmia [22-23]. These properties may offer potential benefits for conditions like cardiovascular diseases and insulin resistance.

2. Materials and Methods

2.1. Collection and authentication of plant

Dried fruits of *Withania coagulance* dunal, (family: *Solanaceae*) were procured from the local market of Pimpri, Pune, Maharashtra, India. Fruit was identified and authenticated by Dr. Randive S.D & Dr. Jagtap M.N, Botanist, herbarium and e-herbarium, Department of Botany and Research centre Solapur, Maharashtra having a specimen no. 3:54-56.1849, wfo00001025156.

2.2. Preparation of plant extract

The dried fruits of *Withania coagulans* dunal purchased from local market, are coarsely powdered. This fruit powder underwent a cold maceration process using an ethanol solvent mixture for a duration of 7 days, resulting in the extraction of the ethanolic extract. To prevent microbial contamination, a few drops of chloroform were added. The extracted mixture was then filtered using muslin cloth and subjected to evaporation at 60ºC until its volume was diminished to one-third of the initial quantity. The resulting filtrate was collected and subjected to evaporation using a rotary evaporator at 40ºC, resulting in a yield of 13.005% w/w. the resulting extract was carefully stored in an airtight container for further use.

2.3. Animals

Male Wistar rats weighing 180-200 gm were used for present study. Theses animals were housed in standard environmental conditions and provided with a standard pellet diet and unrestricted access to water. The study received approval from the institutional animals ethics committee, and sticr adherence to CPCSEA guidelines was maintained throughout the care and experimental process.

2.4. Preliminary phytochemical screening

In our research. We performed preliminary phytochemical screening on the EEWC, revealing the presence of a diverse range of phytoconstituents. Such as steroids, triterpenoids, tannins, flavonoids, alkaloids, carbohydrates was done by qualitative method [24]. Whereas quantitative determination of flavonoids and total phenols was done.

2.4.4. Determination of Total Phenolic Content

The determination of Total Phenolic content (TPC) and total flavonoid content (TFC) was done using modified Folin catechu method and Aluminium chloride method respectively. for TPC 10mg of Gallic acid was dissolved in 10ml of methanol, and various aliquots ranging from 10µg/ml to 50µg/ml were prepared and similarly 10mg of EEWC was dissolved in 10ml methanol, Folin catechu reagent diluted (1:10 v/v) with water. 1ml of test solution EEWC and standard gallic acid dilutions were taken in different test tubes and 1ml of Folin catechu reagent was added to each test tube and 1ml of sodium carbonate solution (7.5 gm/ml) was also added,

the test tubes are kept still in dark for about 30 min and after colour development TPC was analysed using Spectrophotometer at 765 nm [25].

For TFC 10mg of Quercetin was dissolved in 10ml methanol and various aliquots ranging from 10µg/ml to 50µg/ml were prepared and similarly 10mg of EEWC was dissolved in 10ml methanol, 1ml of Test EEWC solution and standard quercetin solution were taken in different test tubes AlCl3 solution (2% v/w) was added to each test tube; mixed and kept still in dark for about 30 min and upon colour development TFC was determined using Spectrophotometer at 420 nm [26].

2.5. Experimental animals and protocol

Male Wistar rats, with an initial weight range of 180-200 gm, were sourced from Crystal Biological Solutions. The rats were accommodated in a temperature-controlled room maintained at 2 2 ± 3ºC, with a 12-hour light-dark cycle, and they had access to water ad libitum. Following one week of acclimatization, the rats were randomly divided into five groups (n= 6/ each group) as given below:

- Normal: control rats received normal water and diet.
- Diseased (DC): rats received (10 % w/v) fructose water ad libitum for 9 weeks.
- DC + Metformin (300 mg/kg): rats received 10% w/v fructose water ad libitum for 9 weeks and 300 mg/kg. b. wt. orally for last 6 weeks.
- DC+ EEWC (200 mg/kg): Rats received (10 %w/v) fructose water ad libitum for 9 weeks and EEWC 200 mg/kg. b. wt. orally for last 6 weeks.
- DC + EEWC (400 mg/kg): Rats received (10 %w/v) fructose water ad libitum for 9 weeks and EEWC 400 mg/kg. b. wt. orally for last 6 weeks.

During the 9 weeks of the experiment, the fructose-drinking groups were given (10% w/v) fructose solution ad libitum, while the normal animals were given regular water. For the final 6 weeks of the trial, treatment groups received *Withania coagulans* dunal fruit extract orally. Weekly body weight measurements were carefully recorded throughout the study. These dietary periods continued for a span of 9 weeks. Sample collection and biochemical analysis. At the conclusion of this study, blood samples were obtained from the retro-orbital plexus while the animals were under anaesthesia induced by a combination of ketamine (100 mg/kg).

The collected blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then preserved in Eppendorf tubes at a temperature of 20 ºC. This stored serum was later utilized for the analysis and evaluation of various biochemical parameters.

2.6. Body weight, blood glucose levels and serum lipid profiles

Animal weight changes were recorded every week until the end of the study. Blood glucose levels were determined by fasting the rats over-night and blood withdrawal by tail vein puncture method, and testing the samples with glucometer every week. Serum analysis was

performed to estimate the levels of total cholesterol, triglycerides, HDL, LDL, and VLDL were determined by auto-analyser (Erba Mannheim test kits).

2.7. Determination of cardiovascular risk indices

1. Castelli risk index I & II (CRI-I & II) were calculated using the formulae presented below.

    1. CRI-I = TC/HDL
    2. CRI-II = LDL/HDL

2. The atherogenic coefficient (AC) was computed with the following.

    1. AC = (TC-HDL)/HDL

3. The expression used to calculate the atherogenic index of plasma (AIP) was.

    1. AIP = log (TG/HDL)

2.8. Determination of inflammatory biomarkers.

Estimation of inflammatory biomarkers such as TNF-α, CRP (C- reactive protein), and homocysteine levels (Hcy) was conducted at preclinbio solutions LLP, located in Pune. Serum level of TNF- α, was evaluated by ELISA kits.

2.9. Histological investigations

After 9 weeks of giving 10 % w/v fructose drinking water and EEWC treatment, all animals were euthanized and their hearts were dissected and further processed for histological testing. The heart tissue were preserved for histological examination by immersing them in 10% formalin buffer solution.

2.10. Statistical analysis

Values are given as mean ± SEM. The results were analyzed by one-way ANOVA test using, GraphPad Prism Version 10.2.3(403) software. This robust statistical approach allows for a comprehensive examination of the data and the identification of significant difference between groups.

3.Results

3.1. Qualitative analysis of EEWC

3.1.1. Preliminary phytochemical testing

The *Withania coagulans* fruit extract showed presence of various phytochemical constituents like alkaloids, steroids, flavonoids, tannins and carbohydrates.

Table 1: Phytochemicals present in EEWC

Test for steroids & triterpenoids	
Salkowski's test	+
Test for tannins	
Lead acetate test	+
Bromine water test	+
Test for flavonoids	
Alkali test	+
Test for alkaloids	
Hager's reagent test	+
Mayer's reagent test	_
Wagner's reagent test	+
Test for carbohydrates	
Fehling's test	+
Benedict's test	+

3.2. Quantitative analysis of EEWC

The percentage of the weight of the sample composition was expressed by phenolic and flavonoids from the EEWC.

3.2.1. Total Phenolic content and total flavonoid content:

The EEWC had a total phenolic content of 5.4808 mg gallic acid equivalent/mg of dry extract and total flavonoid content of 6.56 mg quercetin equivalent/ mg of dry extract.

3.3. Effect of EEWC treatment on Body weight

3.3.1. Body weight

The fructose-drinking control group displayed a noticeable increase in weight at week 9, signifying statistical significance ($p < 0.0001$) when compared to the normal group. Towards the end of the study, the administrations of metformin resulted in a significant reduction in weight compared to the disease control group. Similarly, oral administration of EEWC at doses of 200 mg/kg and 400 mg/kg also induced a significant ($p < 0.01$) decreases in weight when compared to the disease control group. As illustrated in [figure 1].

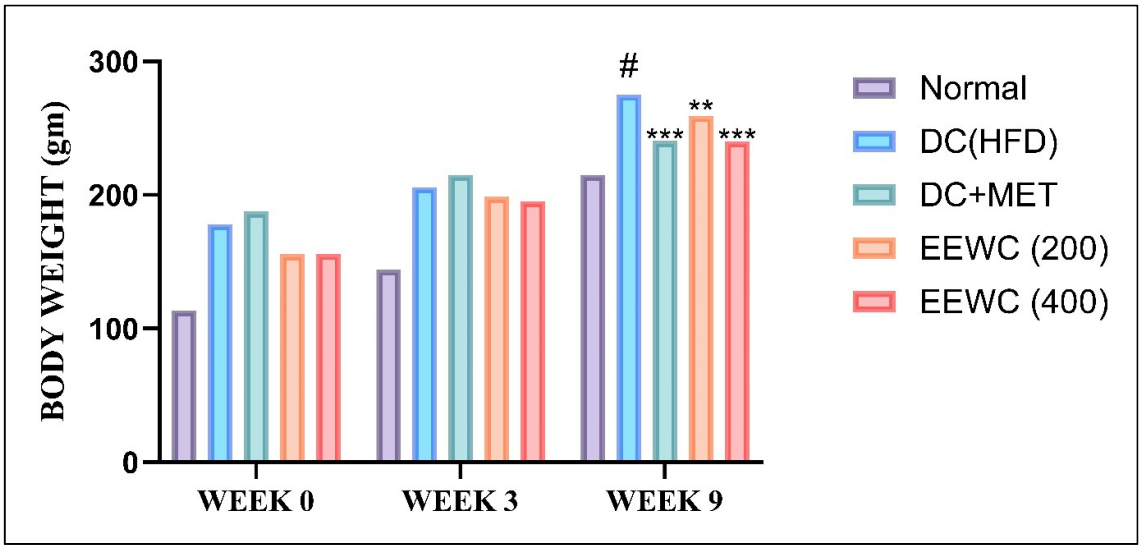

Figure 1: Effect of EEWC on body weight in high fructose-induced metabolic syndrome rats. NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.4. Effect of EEWC treatment on blood glucose and insulin sensitivity

The biochemical parameters encompass fasting blood glucose, serum insulin levels, HOMA-IR.

HOMA-IR is assessed = [fasting insulin (µIU/mL) x fasting glucose (mmol/L)]/22.5.

3.4.1. Fasting blood glucose level.

The fructose-drinking group exhibited a significant increase in fasting blood glucose at week 9, demonstrating statistical significance ($p < 0.0001$) when compared to normal group. In contrast, metformin exhibited a significant ($p< 0.001$) reduction in fasting blood glucose levels in comparison to the normal control group. Furthermore, treatment with EEWC at both doses (200 mg/kg and 400 mg/kg) led to a statistical significance ($p < 0.01$) decrease in fasting bllod glucose levels compared to the disease control group, as illustrated in [figure 2].

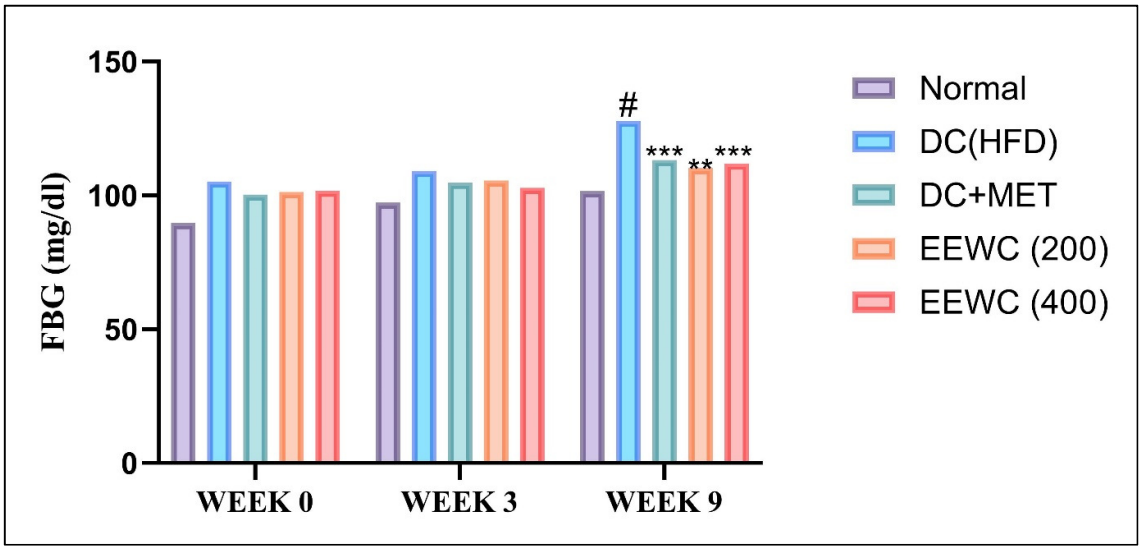

Figure 2: Effect of administration of EEWC on Fasting blood glucose (FBG):NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.4.2. Serum insulin and HOMA-IR

There was a significant increase in serum insulin levels in the insulin-resistant group ($p < 0.0001$) in comparison to the normal control group. Conversely, a remarkable decrease in serum insulin levels was observed in the groups treated with metformin and EEWC at doses of 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p< 0.001$) when compared to the disease control group, and a significant ($p< 0.0001$) increase in HOMA-IR levels was evident in the insulin-resistant group in comparison to the normal control group. Conversely, a substantial decrease ($p < 0.0001$) was observed in the HOMA-IR level of the group treated with metformin. Furthermore, the

administration of EEWC exhibited a significant reduction at doses of 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p < 0.0001$) when compared to the disease control group, as illustrated in [Table 2]

Groups	**Insulin (mg/dl)**	**HOMA-IR**
Normal	1.046667±0.027406	0.283333±0.025517
DC+HFD	2.77±0.207204$^{\#}$	0.978333±0.051602$^{\#}$
DC+MET	1.518333±0.068965***	0.47±0.041553***
DC+EEWC (200mg/kg)	2.416667±0.149257*	0.675±0.046025**
DC+EEWC (400mg/kg)	1.733333±0.156347**	0.476667±0.046019***

Table 2: Effect of administration of EEWC on Insulin sensitivity index; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, *P < 0.05, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.5. Effect of EEWC treatment on lipid profiles

Lipid profiles which include TG, TC, HDL, LDL and VLDL.

3.5.1. Serum triglycerides

The serum triglyceride level in the disease control group displayed a notable increase ($p < 0.0001$) compared to the normal control group. In contrast, a significant decrease ($p < 0.0001$) was evident in the serum triglyceride level of the group treated with metformin. Furthermore, the administration of EEWC at both doses (200 and 400 mg/kg) resulted in a significant ($p < 0.001$) reduction in triglyceride levels when compared to the disease control group, as illustrated in [Figure 3].

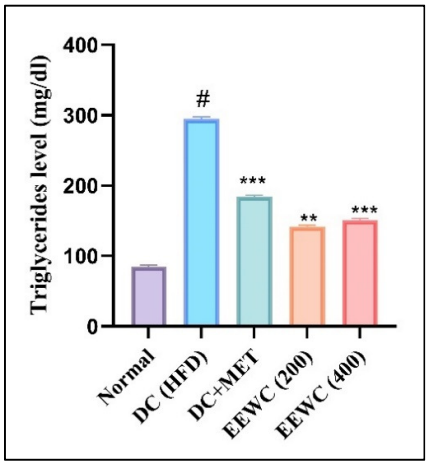

Figure 3: Effect of administration of EEWC on Triglycerides; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.05, **P < 0.001, ***p<0.0001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.5.2. Total cholesterol

The total cholesterol level in the disease control group demonstrated a substantial increase ($p < 0.0001$) in comparison to the normal control group. Conversely, a significant increase ($p < 0.001$) was observed in the cholesterol levels of the group treated with metformin. Notably, the administration of EEWC at doses 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p < 0.001$) effectively led to a significant restoration of total cholesterol levels when compared to the disease control group, as illustrated in [Figure 4].

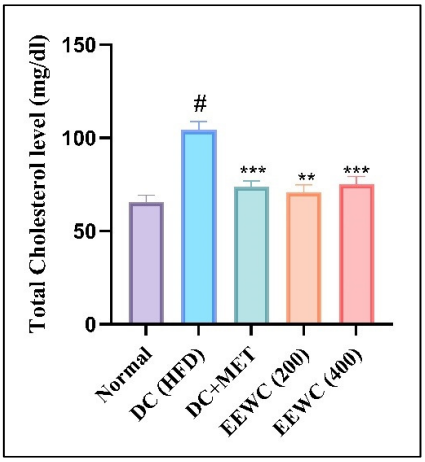

Figure 4: Effect of administration of EEWC on Total cholesterol; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.05, **P < 0.001, ***p<0.0001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.5.3. LDL

When compared to the normal control group, rats in the disease control group exhibited a significant ($p < 0.0001$) elevation in LDL levels. Treatment with metformin resulted in a noteworthy reduction in LDL levels ($p < 0.0001$). Notably, oral administration of EEWC at both doses (200 and 400 mg/kg) significantly ($p < 0.001$) restored LDL levels to normal, as illustrated in [Figure 5].

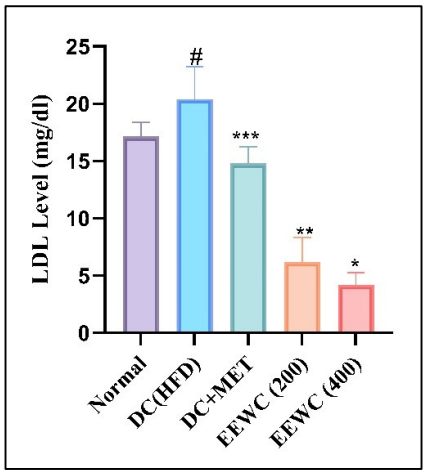

Figure 5: Effect of administration of EEWC on LDL level; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.05, *P < 0.01, **P < 0.001, ***p<0.0001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.5.4. VLDL

In comparison to the normal control group, the disease control group exhibited a significant increase ($p < 0.0001$) in VLDL levels. Conversely, a significant decrease ($p < 0.0001$) was observed in the VLDL levels of the group treated with metformin. Furthermore, treatment with EEWC at both doses (200 and 400 mg/kg) led to a significant ($p < 0.001$) reduction in VLDL levels in comparison to the disease control group, as illustrated in [Figure 6].

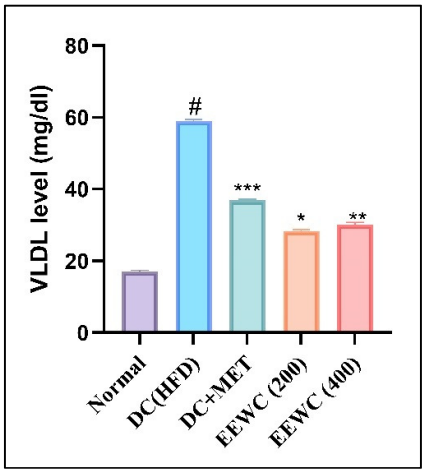

Figure 6: Effect of administration of EEWC on VLDL level; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.05, *P < 0.01, **P < 0.001, ***p<0.0001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.5.5. HDL

The HDL level in the disease control group exhibited a marked decrease ($p < 0.0001$) when compared to the normal control group. In contrast, a significant increase ($p < 0.0001$) was evident in the HDL levels of the group treated with metformin. Furthermore, oral administration of EEWC at both doses (200 and 400 mg/kg) resulted in a substantial ($p < 0.001$) increase in HDL levels when compared to the disease control group, as illustrated in [Figure 7].

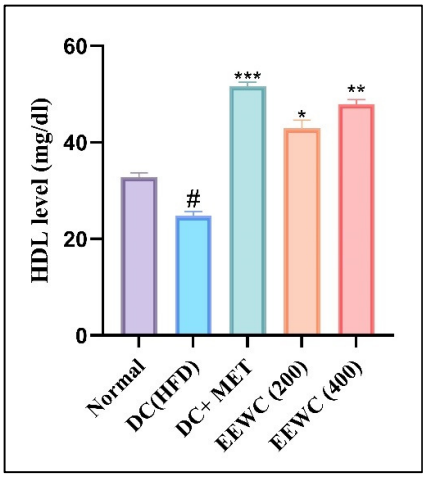

Figure 7: Effect of administration of EEWC on HDL level; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.05, *P < 0.01, **P < 0.001, ***p<0.0001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.6. Effect of EEWC treatment on Liver Enzyme

Liver enzymes which include ALT and AST.

3.6.1. ALT

ALT levels in the disease control group exhibited a significant increase ($p < 0.0001$) when compared to the normal control group. However, oral administration of metformin ($p < 0.0001$) and EEWC at doses 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p < 0.001$) led to a substantial restoration of ALT levels in comparison to the disease control groups, as illustrated in [figure 8].

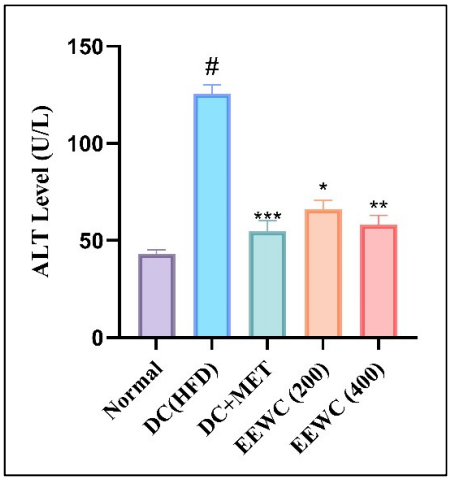

Figure 8: Effect of administration of EEWC on liver enzyme (ALT): NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, *P < 0.05, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.6.2. AST

The AST level in the disease control group displayed a significant increase ($p < 0.0001$) in comparison to the normal control group. Conversely, a significant decrease ($p < 0.0001$) was observed in the AST level of the group treated with metformin. Additionally, treatment with

EEWC at both doses (200 and 400 mg/kg) resulted in a significant ($p < 0.001$) reduction in AST levels when compared to the disease control group, as illustrated in [figure 9].

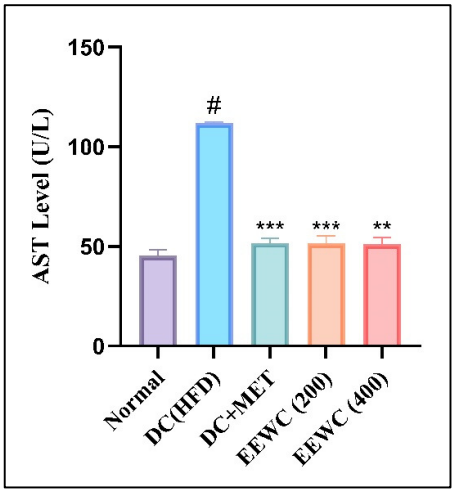

Figure 9: Effect of administration of EEWC on liver enzyme (AST): NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.7. Effect of EEWC treatment on cardiovascular risk indices

Cardiovascular risk indices CRI-I, CRI-II, AC, AIP are explained as follows.

The untreated fructose-drinking group showed significantly higher blood concentrations of TG, TC, LDL, and VLDL, as well as significant drop ($p < 0.001$) in HDL levels. Furthermore, the estimated cardiovascular risk indices Castelli risk index I and II, atherogenic coefficient, atherogenic index of plasma (CRI-I and II, AC, AIP) were considerably greater ($p <0.001$) in this group compared to normal controls. Fructose-drinking groups were treated with EEWC (200 and 400 mg/kg), which repaired the observed lipid abnormalities and reduced CRI-I & II, AC, and AIP indices, as illustrated in [Table 3].

Groups	CRI-I	CRI-II	AC	AIP
Normal	2 ±0.072	0.48±0.072	1±0.079	0.40±0.012
DC+HFD	4.23±0.20$^{\#}$	0.84±0.14$^{\#}$	3.23±0.200$^{\#}$	1.07±0.014$^{\#}$
DC+MET	1.42±0.03***	0.28±0.027***	0.421±0.031***	0.54±0.008***
DC+EEWC (200mg/kg)	1.66±0.09**	0.13±0.047**	0.66±0.09**	0.51±0.015***
DC+EEWC (400mg/kg)	1.56±0.04***	0.083±0.023*	0.56±0.049***	0.49±0.016**

Table 3: Effect of administration of EEWC on cardiovascular risk indices;(CRI-I& II; Castelli risk index I and II, AC; atherogenic coefficient, AIP; atherogenic index of plasma); NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, *P < 0.05, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.8. Effect of EEWC treatment on inflammatory biomarkers

Inflammatory biomarkers such as TNF-α, CRP, homocysteine levels are explained as follows.

The extent of inflammation was assessed by determining TNF-α, CRP, homocysteine levels in serum, TNF-α, CRP, homocysteine levels in serum concentration exceeded normal limits, suggesting a pro-inflammatory cytokine response in high fructose-drinking rats. The TNF-α, CRP, homocysteine level in the disease control group exhibited a significant increase ($p < 0.0001$) compared to the normal control group. Conversely, a significant decrease ($p < 0.0001$) was evident in the CRP level of the group treated with metformin. Furthermore, oral administration of EEWC at doses of 200 mg/kg ($p < 0.001$) and 400 mg/kg ($p < 0.0001$) effectively restored the TNF-α [figure 10], CRP [figure 11], homocysteine level [figure 12] to normal in comparison to the disease control group, as illustrated.

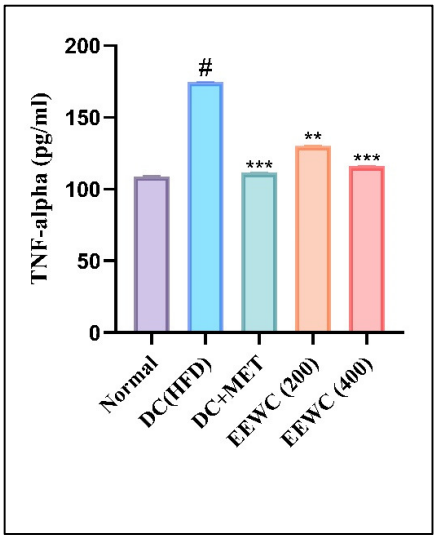

Figure 10: Effect of administration of EEWC on TNF-α; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

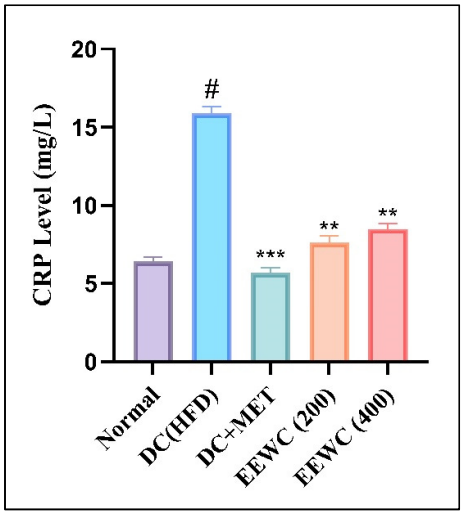

Figure 11: Effect of administration of EEWC on C-reactive protein (CRP); NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

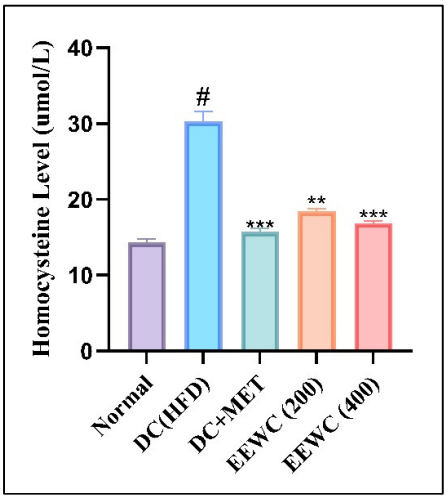

Figure 12: Effect of administration of EEWC on Homocysteine level; NC, normal control; DC, disease control; MET, Metformin; EEWC, ethanolic extract of Withania coagulans (200& 400 mg/kg) fruits**.** Values are expressed as Mean ± SEM. (n =6), #P< 0.0001, **P < 0.01, ***p<0.001; Compared with disease control (ANOVA followed by multiple Dunnet's test)

3.9. Effect of EEWC treatment on Heart Histology

The consumption of high-fructose drinking water led to histological changes in the heart of rats in the High-fructose drinking group, including necrosis, cardiac muscle degeneration. Loss of muscle striations. These findings indicate significant damage to the heart tissue as a result of the high fructose intake. The administration of metformin (300 mg/kg) and EEWC (200 and 400 mg/kg) notably mitigated these alterations, leading to a significant improvement in the histological structure, as illustrated in [figure 8]

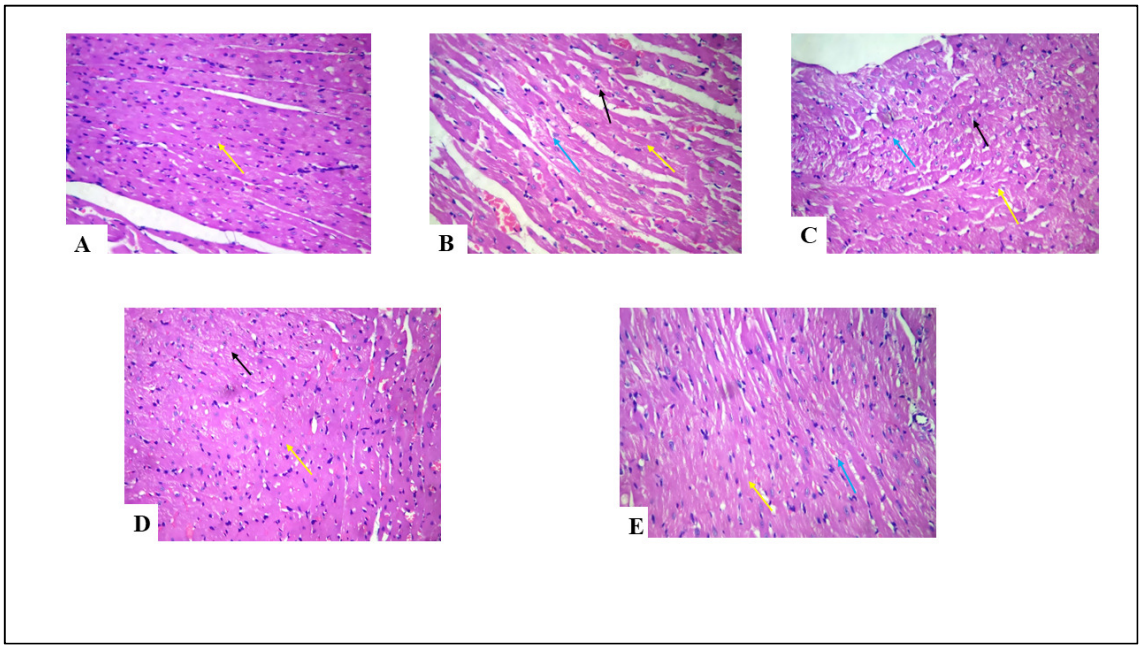

Figure 13: Effect of administration of EEWC on histology of cardiac tissue;

[A] Normal control group; [B] DC(HFD): High fructose-drinking control rats; [C] DC+MET: High fructose drinking rats treated with metformin at 300mg/kg b.w./day; [D] DC+ EEWC (200mg/kg): High fructose drinking rats treated with EEWC extract at 200mg/kg b.w/day; [E] DC+ EEWC (400mg/kg): High fructose drinking rats treated with EEWC extract at 400 mg/kg b.w/day. H&E staining. Yellow arrows: cardiac muscles; blue arrows: cardiac muscle degeneration; black arrows: Loss of Muscle Straiations.

4. Discussion

Fructose intake in human subjects and in experimental animals has been linked to several of the components of metabolic syndrome, such as insulin resistance, glucose intolerance, obesity, and hypertension [27]. Furthermore, when consumed in significant amounts as part of a high-calorie diet, it can cause an increase in total and visceral fat mass, as well as the deposition of ectopic fat in the liver and skeletal muscle [28]. In the current study, after 9 weeks of feeding fructose as liquid solution (10% w/v) to Wistar rats, the fructose drinking group showed substantial increases in body weight [figure 1] and fasting glycemic levels. Moreover, serum insulin concentrations and insulin resistance index (HOMA-IR) were significantly ($p < 0.001$) higher in fructose drinking rats [Table 1] than a normal control animal, demonstrating that

experimental MetS rats have obesity, hyperglycemia, hyperinsulinemia, and insulin resistance, as previously reported in this animal model [29,30,31]. Treating fructose-drinking rats with an ethanolic extract of *W. coagulans* fruits lower FBG by enhancing peripheral glucose consumption [figure2]. Previous phytochemical studies have shown that alkaloids and steroids extracted from different plant sources are responsible for their hypoglycemic action [32]. Significantly reduced body weight, insulin levels, and improved insulin sensitivity.

Fructose is highly lipogenic food linked to the development of severe metabolic dyslipidemia, which appears to be caused by hepatic and intestinal overproduction of atherogenic lipoprotein particles [33,34]. Diabetes mellitus alters lipid metabolism, increasing the mobilization of free fatty acids from muscle and fat accumulation in organs such as the liver and heart [35].

Diabetes dyslipidemia is characterised by increased triglycerides (TG) and reduced high density lipoprotein (HDL), as well as hypercholesterolemia, which is a major risk factor for atherosclerosis and cardiovascular disease [36]. Insulin influences numerous aspects of lipid metabolism, including cholesterol and fatty acid production, via controlling lipid metabolizing enzymes [37]. The EEWC treated MetS rats had considerably lower TG[figure 3], TC[figure 4], and LDL[figure 5] and VLDL[figure 6] levels, as well as higher HDL[figure 7] levels, which may assist to lesson the risk of diabetic complications. Treatment with EEWC also resulted in a considerable decrease in lipid content in the liver, heart, and muscle when compared to MetS animals.

The untreated fructose-drinking group had significantly higher ($p < 0.001$) cardiovascular risk indices (CRI-I, CRI-II, AC and AIP) compared to normal control values due to an increase in lipid profile, including total cholesterol, total triglycerides, LDL and VLDL, and a decrease in HDL levels. The detected lipid abnormalities were reversed after treating the rats with EEWC in a dose-dependent manner, as were the levels of CRI-I, CRI-II, AC, and AIP [Table 3].

As a marker of liver injury, we measured the levels of ALT (alanine transaminase) in the rat that received various treatments. Our findings about ALT levels give important information about the experimental animals' hepatic health. Our findings revealed a significant rise in ALT levels among the group with high-fructose induced MetS. This rise in ALT levels implies liver damage or dysfunction induced by excessive fructose consumption [figure 8]. After administration of EEWC, there was a significant decrease in ALT levels when compared to the MetS group. This data implies that EEWC may have hepatoprotective properties and might potentially reduce liver damage induced by excessive fructose intake. Our findings are

consistent with previous research that has proven the hepatoprotective qualities of EEWC in liver illness due to its antioxidant and anti-inflammatory capabilities, which can help decrease inflammation and oxidative stress in the liver. These qualities may help to restore hepatic function and reduce ALT levels, as seen in our study.

As part of our analysis, we also measured the levels of aminotransferase (AST), a typical marker of liver damage, to assess liver health in our animal model. Our findings suggested that administering a high fructose diet resulted in a significant rise in AST levels. This result suggests hepatocellular damage and liver dysfunction. [figure 9] The therapy with EEWC considerably reduced the rise in AST levels caused by excessive fructose intake. These findings imply that the extract has a hepatoprotective effect. This impact may be due to EEWC's capacity to limit lipid buildup, improve lipid metabolism, and reduce oxidative stress in hepatocytes.

High fructose consumption resulted to elevated levels of pro-inflammatory cytokines (TNF-α) and markers (CRP). The observed aberrations in these evaluated parameters might be attributed to the rats' greater body mass, as seen by higher obesity index and visceral fat content. This view was supported by the relatively recent discovery that adipose tissue, particularly visceral fats, is the primary source of cytokines that act as significant mediators in the inflammatory state of MetS [38]. Emerging scientific and clinical data has revealed as a robust link between CRP and several MetS characteristics. Furthermore, Ridker et al. [39] proposed that CRP be used as clinical criteria of MetS [figure 11]. TNF- α may activate inflammatory pathways including NF-κB and block insulin signaling, leading to insulin resistance, type 2 diabetes, and cardiovascular events [40]. In our investigation, the rats in the fructose consuming group had considerably higher CRP levels. This suggest the existence of systemic inflammation, which is associated with insulin resistance and the development of heart muscle dysfunction. Increased CRP and TNF- α levels may indicate activation of pro-inflammatory pathways, including the NF-κB signaling pathway, which regulates inflammatory response [figure 10]. We found that after treating the animals with EEWC, CRP levels decreased significantly. These findings imply that EEWC has inflammatory capabilities that might help reduce the inflammatory response, ultimately leading to a drop in CRP (C-reactive protein) production.

Many people's CVD cannot be fully explained by established risk factors like hypertension and high blood cholesterol. For example, 35% of coronary heart disease instances occur in patients with total cholesterol levels < 200 mg/dl. Other risk variables, such as blood

homocysteine concentrations, appear to be strongly associated with CVD. This section explores the data supporting homocysteine as an independent risk factor for CVD, explains the putative mechanisms via which homocysteine contributes to CVD, and investigates the levels of blood homocysteine concentrations linked with increased CVD risk [41]. The hyperhomocysteinemia (HHcy) has seen in the fructose fed model of IR might be related to the reduction in the specific activity of two major enzymes of homocysteine (Hcy) metabolism, namely, methyltetrahydrofolate reductase and cystathionine β synthase (CβS). Diker-Brown et al. employed cultured hepatocytes to demonstrate that continuous insulin addition might create HHcy, which was owing to Hcy being converted to methionine or cysteine at a slower rate. HHcy might potentially be explained by the reported hypertriglyceridemia, which may enhance lipid accumulation in visceral adipose tissue, as is prevalent with IR. N-nicotinamide methyltransferase (NNMT) is a prominent methyltransferase found in human adipose tissue. It transforms nicotinamide to N-methyl nicotinamide at the expense of S-adenosyl methionine, the methyl-donating cofactor. The produced S-adenosyl homocysteine might then be transformed to Hcy. Thus, the observed HHcy in the current study's fructose-drinking rat model might be due to increased visceral adiposity associated with fructose overconsumption [42]. [figure 12] In our investigation, the rats in the fructose consuming group had considerably higher Hcy levels. This demonstrates the likelihood of atherosclerosis disease in animals fed fructose. We found that when the animals were treated with EEWC, their homocysteine levels dropped significantly.

This study looked at cardiac tissue histopathology in rats with high fructose drinking metabolic syndrome.

High fructose consumption caused lipotoxicity with higher systemic TG levels, which increased intramyocellular TG content in the cardiac muscle, resulting in IR, which is associated with contractile dysfunction and an increased risk of developing hypertension and atherosclerotic heart disease. Histological results of cardiac tissue in high fructose drinking-induced metabolic syndrome revealed degenerative alterations such as necrosis and fibrosis, as well as the deposition of fat globules in myocardial cells, with substantial fatty changes in the portal area as compared to the control group. [figure 13] Metformin decreased cardiac muscle degeneration, whereas EEWC at 200 mg/kg showed some improvement. At 400 mg/kg, EEWC significantly reduced cardiac tissue damage such as cardiac muscle degeneration, muscle striation loss, and necrosis, most likely due to its anti-inflammatory properties, implying that EEWC may have a cardioprotective effect on high fructose-induced metabolic syndrome

through lipid metabolism modulation, oxidative stress reduction, and inflammation suppression.

5. Conclusion

Based on the findings and observations from the current study, it is concluded that *Withania coagulans* dunal fruit extract is useful in the treatment of high fructose induced metabolic syndrome. The extract of *Withania coagulans* dunal fruit has a positive effect on cardioprotective activities, notably in correcting hyperlipidemia and enhancing insulin sensitivity. The presence of antioxidants in EEWC may be responsible for its cardioprotective action, which contributes to its positive effects on the heart tissue. The presence of phenols, flavonoids, withanolides and other secondary metabolites may account for EEWC extracts high activity.

6. Acknowledgement

The authors extend their heartfelt appreciation to their mentor, as well as to the Management and Principal of P.E.S. Modern College of Pharmacy, Nigdi, Pune, for their generous provision of the necessary facilities to carry out this research**.**

7. References:

1. Grundy SM, Hansen B, Smith Jr SC, Cleeman JI, Kahn RA, Conference Participants. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. Circulation. 2004 Feb 3;109(4):551-6.
2. Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, Deng L, Bry L, Gordon JI, Kahn CR. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell metabolism. 2015 Sep 1;22(3):516-30.
3. Grundy SM. Metabolic syndrome pandemic. Arteriosclerosis, thrombosis, and vascular biology. 2008 Apr 1;28(4):629-36.

4. Kim HY, Okubo T, Juneja LR, Yokozawa T. The protective role of amla (Emblica officinalis Gaertn.) against fructose-induced metabolic syndrome in a rat model. British journal of nutrition. 2010 Feb;103(4):502-12.
5. Arozal W, Louisa M, Soetikno V. Selected Indonesian medicinal plants for the management of metabolic syndrome: Molecular basis and recent studies. Frontiers in cardiovascular medicine. 2020 May 6;7:82.
6. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary and alternative medicines. 2013 Aug 14;10(5):210-29.
7. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. 2. Dehradun, India. Bishen mahendra pal singh. 1975:842-4.
8. Negi MS, Sabharwal V, Wilson N, Lakshmikumaran MS. Comparative analysis of the efficiency of SAMPL and AFLP in assessing genetic relationships among Withania somnifera genotypes. Current science. 2006 Aug 25:464-71.
9. Gupta V, Keshari BB. Withania coagulans Dunal (paneer doda): A review. International Journal of Ayurvedic and Herbal Medicine. 2013;3(5):1330-6.
10. Ullah Z, Baloch MK, Khader JA, Baloch IB, Ullah R, AbdEIslam NM, Noor S. Proximate and nutrient analysis of selected medicinal plants of Tank and South Waziristan area of Pakistan. Afr. J. Pharm. Pharmacol. 2013 Feb 8;7(5):179-84.
11. Ram H, Kumar P, Purohit A, Kashyap P, Kumar S, Kumar S, Singh G, Alqarawi AA, Hashem A, Abd-Allah EF, Al-Arjani AB. Improvements in HOMA indices and pancreatic endocrinal tissues in type 2-diabetic rats by DPP-4 inhibition and antioxidant potential of an ethanol fruit extract of Withania coagulans. Nutrition & Metabolism. 2021 Dec;18:1-7.
12. Qasim S, Zafar A, Saif MS, Ali Z, Nazar M, Waqas M, Haq AU, Tariq T, Hassan SG, Iqbal F, Shu XG. Green synthesis of iron oxide nanorods using Withania coagulans extract improved photocatalytic degradation and antimicrobial activity. Journal of Photochemistry and Photobiology B: Biology. 2020 Mar 1;204:111784.
13. Peerzade N, Sayed N, Das N. Antimicrobial and phytochemical screening of methanolic fruit extract of Withania coagulans L. Dunal for evaluating the antidiabetic activity. Pharma Innov. J. 2018;7:197-204.
14. Qureshi SA, Jahan M, Lateef T, Ahmed D, Rais S, Azmi MB. Presence of gallic acid and rutin improve the hepatoprotective strength of Withania coagulans. Pakistan Journal of Pharmaceutical Sciences. 2019 Jan 2;32.
15. Lateef T, Qureshi SA. Centratherum anthelminticum and Withania coagulans improves lipid profile and oxidative stress in triton X-100 induced hyperlipidemic rabbits. Group. 2020;1(11.36):1-22.
16. Keshari AK, Srivastava A, Upadhayaya M, Srivastava R. Antioxidants and free radicals scavenging activity of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. 2018;7(3):1499-504.
17. Ahmad R, Fatima A, Srivastava AN, Khan MA. Evaluation of apoptotic activity of Withania coagulans methanolic extract against human breast cancer and Vero cell lines. Journal of Ayurveda and integrative medicine. 2017 Jul 1;8(3):177-83.
18. Gosavi DD, Kamdi AS, Kalambe SM, Bohra PN. The motor coordination activity of alcoholic extract of Withania coagulans fruits in Swiss albino mice by rota rod test. Indian J. Pharm. Pharmacol. 2020;7:73-6.

19. Mirakzehi MT, Hosseini SJ, Saleh H. The effects of hydroalcoholic extracts of Withania somnifera root, Withania coagulans fruit and 1, 25-dihydroxycholecalciferol on immune response and small intestinal morphology of broiler chickens. Journal of Applied Animal Research. 2017 Jan 1;45(1):591-7.
20. Reddy SS, Chauhan P, Maurya P, Saini D, Yadav PP, Barthwal MK. Coagulin-L ameliorates TLR4 induced oxidative damage and immune response by regulating mitochondria and NOX-derived ROS. Toxicology and Applied Pharmacology. 2016 Oct 15;309:87-100.
21. Maurya R. Chemistry and pharmacology of Withania coagulans: an Ayurvedic remedy. Journal of pharmacy and pharmacology. 2010 Feb;62(2):153-60.
22. Mudassir HA, Nazim K, Khan UA, Qureshi SA. Comparative evaluation of hypoglycemic activity, trace minerals and phytochemical contents of some potential medicinal plant extracts. Int. J. Biol. Biotechnol. 2018 Apr 25;15:55-62.
23. Saratha R, Kas MS, Sundari KK. Evaluation of anti-helminthic activity of ethanolic extract of Withania coagulans. Res. J. Pharm. Sci. 2019;8:555X.
24. Khandelwal KR. Practical Pharmacognosy. 12thbEd. Nirali Prakashan, Pune. 2004:149-56.
25. Kulshreshtha A, Saxena J. Qualitative and quantitative estimation of phyto constituents in different solvent extracts of leaf of Tabernaemontana divaricata. Journal of Pharmacognosy and Phytochemistry. 2022;11(4):45-50.
26. Baraskar, S.; Saxena, J. Screening of phytochemicals and in vitro antimicrobial activity hydroalcoholic extract of gardenia resinifera. Eur. Chem. Bull., 2023, 12, 1757-1762.
27. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome. American Journal of Physiology-Endocrinology and Metabolism. 2010 Nov 1.
28. Tappy L, Lê KA, Tran C, Paquot N. Fructose and metabolic diseases: new findings, new questions. Nutrition. 2010 Nov 1;26(11-12):1044-9.
29. Mohan M, Khade B, Shinde A. Effect of A-HRS on blood pressure and metabolic alterations in fructose-induced hypertensive rats. Natural product research. 2012 Mar 1;26(6):570-4.
30. Mohan M, Khade B, Shinde A. Effect of A-HRS on blood pressure and metabolic alterations in fructose-induced hypertensive rats. Natural product research. 2012 Mar 1;26(6):570-4.
31. Réggami Y, Benkhaled A, Boudjelal A, Berredjem H, Amamra A, Benyettou H, Larabi N, Senator A, Siracusa L, Ruberto G. Artemisia herba-alba aqueous extract improves insulin sensitivity and hepatic steatosis in rodent model of fructose-induced metabolic syndrome. Archives of physiology and biochemistry. 2021 Nov 2;127(6):541-50.
32. Adebajo AC, Ayoola OF, Iwalewa EO, Akindahunsi AA, Omisore NO, Adewunmi CO, Adenowo TK. Anti-trichomonal, biochemical and toxicological activities of methanolic extract and some carbazole alkaloids isolated from the leaves of Murraya koenigii growing in Nigeria. Phytomedicine. 2006 Mar 13;13(4):246-54.
33. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutrition & metabolism. 2005 Dec;2:1-4.
34. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome. American Journal of Physiology-Endocrinology and Metabolism. 2010 Nov 1.

35. Bloomgarden ZT. Fat metabolism and diabetes: 2003 American diabetes association postgraduate course. Diabetes care. 2003 Jul 1;26(7):2198-203.
36. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature Reviews Endocrinology. 2009 Mar;5(3):150-9.
37. Shukla K, Dikshit P, Tyagi MK, Shukla R, Gambhir JK. Ameliorative effect of Withania coagulans on dyslipidemia and oxidative stress in nicotinamide–streptozotocin induced diabetes mellitus. Food and Chemical Toxicology. 2012 Oct 1;50(10):3595-9.
38. Waki H, Tontonoz P. Endocrine functions of adipose tissue. Annu. Rev. Pathol. Mech. Dis.. 2007 Feb 28;2(1):31-56.
39. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. New England journal of medicine. 2000 Mar 23;342(12):836-43.
40. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature. 2002 Nov 21;420(6913):333-6.
41. Joubert LM, Manore MM. Exercise, nutrition, and homocysteine. International journal of sport nutrition and exercise metabolism. 2006 Aug 1;16(4):341-61.
42. El Mesallamy HO, El-Demerdash E, Hammad LN, El Magdoub HM. Effect of taurine supplementation on hyperhomocysteinemia and markers of oxidative stress in high fructose diet induced insulin resistance. Diabetology & metabolic syndrome. 2010 Dec;2:1-1.