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Bioflavonoid hesperidin possesses the anti-hyperglycemic and hypolipidemic property in STZ induced diabetic myocardial infarction (DMI) in male Wister rats

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HIGHLIGHTS

- Isolation and characterization of hesperidin from Citrus sinensis (L.) peels.
- Hi-fat diet induces the increased lipid profiles in diabetic rats.
- Hesperidin decrease plasma insulin and lipid profiles in myocardial infarction (DMI) rats.

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Keywords:
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ABSTRACT

The aim of our study was to evaluate the hesperidin anti-hyperglycemic and hypo-lipidemic effects on diabetic myocardial infraction (DMI) rats by decreasing the blood glucose and blood cholesterol contents. The object of the study was to examine the 7groups of male Wistar rats, each group contains 6 rats, Group I (normal), Group II diabetic (control) and 5(experimental) groups, i.e., Group III (diabetic + hesperidin), Group IV (diabetic + Glibenclamide), Group V (ISO), Group VI (diabetic + ISO) and Group VII (diabetic + ISO + hesperidin). By that intake of hesperidin regulates the lipid and carbohydrate metabolism by decreasing the cholesterols in blood and plasma insulin by hyperlipidamic and anti-hyperglycemic activity. The rats turns to diabetic with single intraperitonial STZ injection (50 mg/kg BW), and from the second week the rats diet were switched to a high fat diet, i.e., cholesterol (40%), fat (50%), Protein (50%) turns to be hyperlipidamic rats and with Isoproterenol (ISO) single intraperitoneal injection by the (11 mg/kg BW) turns to DMI rats. The DMI rats fed with the hesperidin 100mg/kg BW for 4 weeks had significantly reduced the blood glucose ($P < 0.05$), total cholesterol (TC), Triglycerides, HDL, LDL and VLDL concentrations, when compared with experimental groups ($p < 0.05$). A significantly increased blood glucose and body weight was observed in hesperidin treated diabetic groups ($P < 0.05$) by comparing with the experimental groups. In conclusion, orally supplementation of hesperidin possesses a significant decrease in total blood lipid profiles and plasma insulin concentrations accompanied by the anti-hyperglycemic, hypo-lipidemic activity in DMI rats.

1. Introduction

Diabetes related cardiomyopathy disease like myocardial infraction (DMI) is the major causes of mortality in western countries and in the Asia-Pacific region. Insulin resistance is commonly associated with obesity and a key factor mediating the progression to T2DM. Failure of insulin-sensitive peripheral tissues to respond to insulin results with an increases serum glucose levels, that leads to an impaired homeostatic state. The skeletal muscle plays a crucial role in maintaining glucose metabolism with proper carbohydrate pathways. Impairments in both glucose and lipid metabolism arising wide variety of adverse actions like dysregulation of hormones, elevated free fatty acid (FFA), or other factors contribute significantly to the pathogenesis of T2DM [1–3]. The elevated FFA in diabetic obesity [4,5], providing evidence that availability of excess fat deposition in the form of FFA may lead to impairments in muscle glucose metabolism and storage, and consequently to glucose intolerance switched to T2DM [5]. Lipoproteins are complexes of lipids and proteins that are essential for the transport of cholesterol,

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triglycerides, and fat-soluble vitamins. Until recently, lipoprotein disorders were the preview of lipidologists, but their demonstrations like that the lipid-lowering therapy significantly reduces the clinical complications of diabetic cardiovascular disease (DCVD) has brought the diagnosis and treatment of these disorders into the domain of the general internist. Furthermore, elevated serum FFA can interfere with glucose utilization both *in vitro* and *in vivo* [6,7]. Together, this evidence implicates disturbed lipid metabolism in the pathogenesis of T2DM obesity is also associated with cardiovascular disease (CVD). Obesity and being overweight is also associated with adiposity is the portion of total body mass that is comprised of neutral lipid deposits in adipose tissues. A strong correlation has been shown to exist between adiposity and insulin resistance leading to T2DM [8–10]. In increased release of high amounts of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines is a hallmark of obesity [10]. Recent evidences regarding the wide range biological activities of hesperidin the isolated compound of *Citrus sinensis* (L.) orange peel extract contains a rich source of flavonoids, glycosides, polymethoxy flavonoids showing promising antioxidant activity in may diseases reported [11]. An attempt has made to find the effect of hesperidin anti-hypoglycemic and hypolipidamic activity in isoproterenol induced DMI rats. The diabetic rats fed a high fat diet cholesterol-containing diet by evaluating the plasma lipid profile and plasma insulin levels and prolong diabetic conditions and single IP injections turns the rats to myocardial infarction (MI) rats. Considering the facts that a) DMI at least in part targets pro and antioxidant status in lipid profiles b) Hesperidin is widely used to treat DMI, the present study was aimed to investigate the effect of hesperidin on antioxidant enzyme levels of controlling the hyperglycemic condition and lipid profiles in the diabetic myocardial rats of induced isoproterenol.

2. Materials and methods

Male albino Wistar rats, aged 3 months old (180 ± 20 g body weight) were used for the present study, we procured animals from Indian Institute of Science, Bangalore, India. All the animals were maintained under regulatory laboratory conditions (12L: 12D; Humidity: 76% and temperature: 28 ± 20° C) in the Department of Zoology, Sri Venkateswara University, Tirupati. The animals maintained under regulated laboratory conditions in polypropylene cages (as per Institutional Animal Ethical Committee, Sri Venkateswara University (No.01/2011–2012/(i)/a/CPCSEA/IAEC/SVU/MB-SSR/Dt 20/06/2011), Tirupati, for four weeks of study provided the standard rat pellet and water *ad libitum*. After acclimatization for 7–10 days, the rats were fasted for 12 h before single intraperitoneal injection of streptozotocin (STZ) (Sigma-Aldrich, 50 mg/kg body weight) dissolved in citrate buffer at pH 4.5. Two days after STZ treatment, the rats were considered diabetic (as determined by non-fasting blood glucose levels of N 140 mg/dL and positive blood glucose test (accucheck). After induction of diabetes, the rats were maintained on a high-fat diet for the duration of the experiment to induce hypercholesterolemia, after acclimatization of 7 days, normal rats diet were switched to a high fat diet a modified NIN 78 diet (National Institute of Nutrition, India) consisting of 50% carbohydrates 14% fats, 50% protein, 46% fiber, and 40% cholesterol by weight, as previously described. Normal rats were fed the same diet without cholesterol, as shown in (Table 1). The rats were maintained on their respective diets for 4 weeks to induce hypercholesterolemia and then for the whole experimental period.

2.1. Isolation of hesperidin

Citrus sinensis (L.) fruits were purchased from the local market of Tirupati, The taxonomic identification of *Citrus sinensis* (L) plant was confirmed by a senior botanist and Asst. Professor. K. Madhava Chetty, Department of Botany, S.V. University, voucher specimen (Herbarium Accession No: 2192) was deposited in the herbarium. The *Citrus sinensis* (L.) fruits peels were separated from fruits, shade dried and cut into small bits and keeping them in Soxhle with hexane as solvent. Then compound was concentrated it by using rotary vapor and material dried was collected. After the orange peels have been completely de-fatted, extract the material remaining in the flask for soaked with methanol and the extract were concentrating under the reduced pressure then washed with distilled water and re-crystallized the residue with aqueous acetic acid. The flavonoid glycoside, hesperidin, colorless needles were separated and used for the investigation.

Table 1
Ingredient composition of the NIN-77 diet with or without added cholesterol. [A] Mineral and Vitamin mixtures.

S.No	Ingredient	Composition (g/100 g)	
		Control	HI-fat diet
1.	Starch	60.0	60.0
2.	Peanut oil	10.0	10.0
3.	Cellulose	1.0	1.0
4.	Vit mix[A]	1.0	1.0
5.	Min mix[A]	1.0	1.0
6.	Casein	40.0	40.0
7.	Sucrose	50.0	55.0
8	Cholesterol	–	40.0

2.2. Hesperidin characterization through FT-IR

The hesperidin is characterized by the FT-IR (Fourier Transform Infrared spectroscopy) analytical technique. FT-IR spectra of the complex were compared to the physical mixture and pure substances ones. Changes in the characteristic bands of pure substances confirm the existence of the complex as a new compound with different spectroscopic bands. FTIR Spectrum of hesperidin in the region (1000-4000 cm^{-1}) was studied.

2.3. Experimental design

Total 42 Rats were randomly divided and assigned 7 groups accordingly normal, diabetic control, and experimental groups, with each group consisting of n = 6 animals. The untreated group (non-diabetic) rats consisted of normal. The experimental control group (diabetic) consisted of STZ (50 mg/kg bw, Intraperitoneal (IP). single injection of STZ) diabetic rats and 5 (experimental) groups diabetic + hesperidin (100 mg/kg bw of Hesperidin), diabetic + glibenclamide (600 µg/kg bw of Glibenclamide), (isoproterenol) ISO (Received 11 mg/kg bw of ISO on 29th and 30th days of experimental period), diabetic + ISO, diabetic + ISO + hesperidin all the animals fed with chow diet first 1 week then switched to high fat diet daily for 4 weeks to turn hypercholesterolemic rats. Total blood cholesterol, TGs, and phospholipids levels were determined using commercial kits.

2.4. Effect of hesperidin on blood glucose levels in diabetic rats

The blood glucose levels in normal and experimental groups were checked at different time intervals. The level of blood glucose was significantly increased in diabetic untreated rats. Oral administration of peel extract of hesperidin, and glibenclamide to diabetic rats significantly reversed all these changes to near normal levels. Hesperidin extract to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of lipid metabolism close to normal values (Fig. 2).

2.5. Biochemical assays

The blood serum total lipid content was estimated and treated with Triton X and used analysis for Triglycerides by using commercial kits

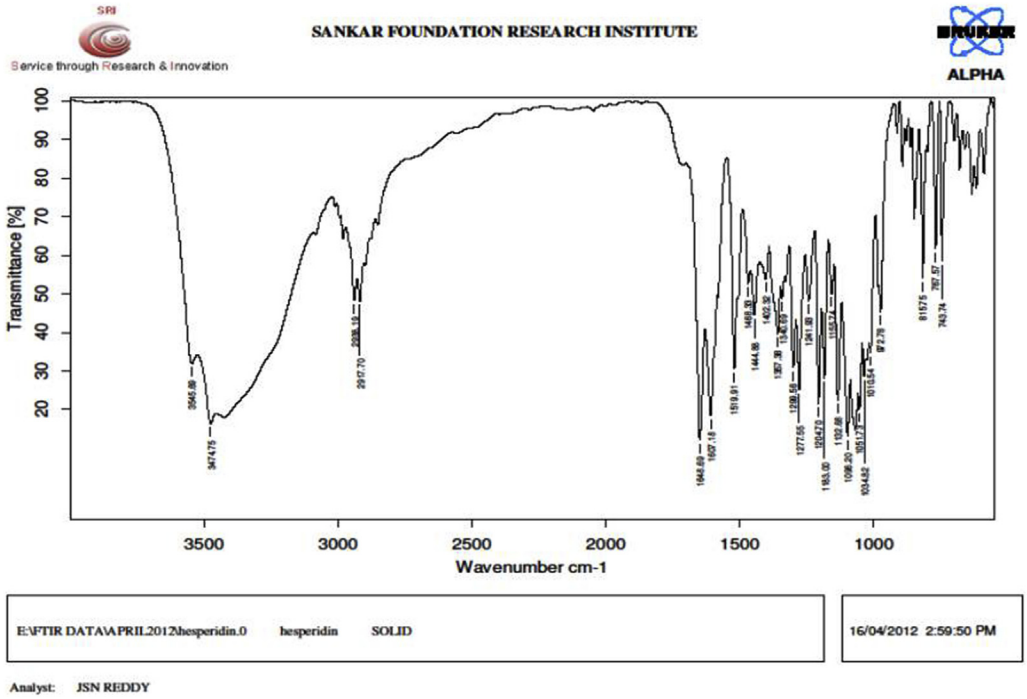

Fig. 1. Shown FTIR spectrum of Hesperidin in the region from 4000 to 1000cm-1.

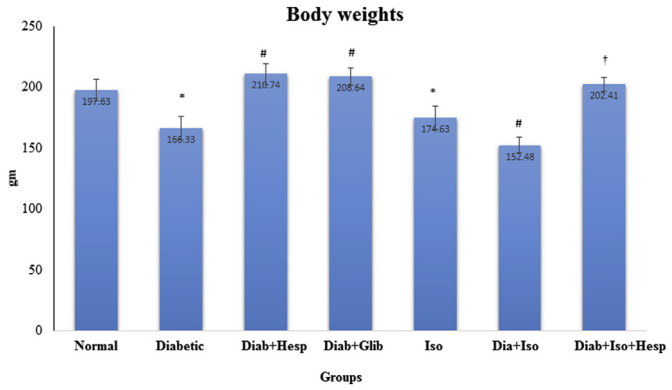

Fig. 2. Effect of hesperidin on body weights changes in Normal, Diabetic, diabetic treated hesperidin (Diab + Hesp), diabetes treated glibenclimade (Diab + Glib), Isoproterenol (Iso), diabetes induced isoproterenol (Diab + Iso), and diabetes induced isoproterenol treated hesperidin myocardial infarction (MI) rats (Diab + Iso + Hesp).

(M/s Excel Diagnostic Pvt. Ltd). And the total cholesterol content was estimated by using the kit method (Medsource Ozone Biomedicals Pvt. Ltd) and low density lipoproteins (LDL) and high density lipoproteins (HDL) concentrations were estimated by using direct enzymatic method of the kits (Coral Clinical Systems, Goa, India) protocols by comparing the standard values and the statistical analysis. The lipid profiles and the cardiac risk factor were calculated using the following formulas.

2.6. Triglycerides

$$Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{GK} G-3-P + ADP$$

$$3-G-P+O_2 \xrightarrow{GPO} H_2O + Dihydroxyacetone\ phosphate$$

$$H_2O_2 + ESPAS + 4-AAP \xrightarrow{POD} Brown\ Color + H_2O$$

2.7. Total cholesterol

$$Cholesterol\ esters + H_2O \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ Oxidase} 4-Cholestenone + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{Amino antipyrine} \xrightarrow{POD} \text{Red Quinoneimine Complex} + H_2O_2$$

2.8. Low-density lipoprotein (LDL)

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ oxidase} 4-\text{Cholestenone} + H_2O_2$$

$$Cholesterol + H_2O \xrightarrow{Cholesterol\ esterase} Cholesterol + Fatty\ acids$$

$$2H_2O_2 + TODS \xrightarrow{Peroxides} Quinonimine + H_2O_2 + 4-\text{Aminiantipyrine}$$

2.9. Statistical analyses

The data were subjected to statistical analysis, such as mean, standard deviation and analysis of variance (ANOVA) using standard statistical software, SPSS (version 16) software. All values are expressed as Mean ± SD of 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

3. Results

3.1. Hesperidin FTIR characterization

The FT-IR spectroscopic analysis confirmed the interaction and the complex formation within the compound.

3.2. Crystalline hesperidin is known to have absorbed at

Very strong-1649 cm^{-1}
Weak- 3545-2938 cm^{-1}
Very weak-2917, 2850 cm^{-1}

Crystalline hesperidin spectrum and their presence in the present FTIR indicates that hesperidin is present in the extracted compound. FTIR spectrum of hesperidin in the region from 4000 to 1000 cm^{-1} is shown. There are fairly sharp peaks at 3545, 2938, 2917, 2850, 1649 cm^{-1} which indicate the presence of the flavonoid compound in the drug. The IR spectra of Hesperidin exhibit the characteristic patterns of flavonoids (experimental part) that generate bands, i.e. the aromatic at 1649(C═O-valence) cm-^{1}, the −OH phenolic at 3545 (O−H-valence), 2938 (C−H-valence, arene), 2917 (C−H-valence, alkane), 2850–2865 (C−H-valence, alkane (OCH3) the methoxylic at 1277 cm-^{1} appear in the IR spectrum of complex as shown in (Fig. 1), while the intensity of the band relative to the carboxylic group decreases.

3.3. Body weights

Hesperidin possesses the body weight (BW) gain nutritional characteristics of diabetic rats when rats fed with and control rats were similar (Table 2). Daily food intakes of hesperidin-fed rats were lower than those of control rats (2.104) but higher than those of normal rats (−3.204). The food efficiency ratios representing body weight gain relative to food intake were the same for both hesperidin-fed and control rats, but their ratios (−61.90) were significantly lower than that observed in normal rats (Table 2). Daily food intakes of diabetic rats fed hesperidin was significantly reduced compared with those of diabetic control rats (−295.84), whereas water intakes for both groups were significantly increased compared with normal rats (295.84). Body weights, food intakes, and food efficiencies were similar to hesperidin and control hypercholesterolemic rats (Table 2). Except for lower food intakes, body weights and food efficiency ratios in hypercholesterolemic rats were significantly higher than those in normal rats. However, hypercholesterolemic rats had higher body weights than normal rats, but those fed hesperidin had lower body weights than control rats (6.63) (Fig. 2) were observed in all experimental groups.

3.4. Blood glucose

The blood glucose levels in normal and experimental groups were checked at different time intervals. The level of blood glucose was significantly increased in diabetic untreated rats. Oral administration of hesperidin, to diabetic rats significantly reversed all these changes to near normal levels. Bioactive compound hesperidin to diabetic animals has been shown to lower blood glucose levels and partially restore the activity of key enzymes of carbohydrate and lipid metabolism close to normal values (Table 3 & Fig. 3).

3.5. Lipid profiles

Ingestion of hesperidin significantly reduced serum cholesterol concentrations by hypolidemic activity in STZ-induced diabetic rats compared with control diabetic rats (Table 4). No significant difference in the Triglycerides (TG) levels among the groups was observed. However, diabetic rats fed hesperidin had significantly lower plasma TG levels than those in control diabetic rats ($P < 0.05$). On the other hand, oral gavage of hesperidin (41.95) and glibenclamide treatment (28.43) compared to diabetic rats significantly decreased. Hypercholesterolemic rats administered hesperidin had significantly lower total plasma cholesterol and LDL levels compared with control rats. In STZ treated ISO diabetic rats, the levels of total cholesterol in the blood were significantly increased (166.09) as compared to control rats. The

Table 2
The effect of hesperidin on body weights observed in all experimental groups normal (N), STZ induced diabetic (D), diabetic treated hesperidin (D + H), diabetes treated glibenclimade (D + G), Isoproterenol alone induced (I), diabetes induced isoproterenol (D + I), and diabetes induced isoproterenol treated hesperidin myocardial infarction (MI) rats (D + I + H).

	Normal (N)	Diabetic (D)	Diabetic + Hesperidin (D + H)	Diabetic + Glibenclamide (D + G)	Isoproterenol (I)	Diabetic + Isoproterenol (D + I)	Diabetic + Isoproterenol + Hesperidin (D + I + H)
Changes in initial body weights (g)	185.37 ± 5.14	189.27^{ns} ± 7.15 (2.104)	179.43^{ns} ± 8.47 (−3.204)	184.16^{ns} ± 3.75 (−0.654)	189.35^{ns} ± 9.14 (2.147)	183.31^{ns} ± 6.81 (−1.111)	186.64^{ns} ± 7.18 (0.685)
Changes in final body weights (g)	197.63 ± 8.67	166.33* ± 9.34 (−15.86)	210.74$^{\#}$ ± 8.45 (6.63)	208.64$^{\#}$ ± 6.86 (5.57)	174.63* ± 9.58 (−11.64)	152.48$^{\#}$ ± 6.46 (−22.86)	202.41† ± 5.62 (2.42)

Values are mean and ± S.D of six rats in each group, ns = non-significant.
Values in parentheses are the percent change of control.
$^{*}p < 0.01$ as compared with normal rats; $^{\#}p < 0.01$ as compared with diabetic rats; $^{\dagger}p < 0.01$ as compared with Diabetic + Isoproterenol.

Table 3
Effect of Hesperidin on serum blood glucose levels (mg/dl) in in all experimental groups normal (N), STZ induced diabetic (D), diabetic treated hesperidin (D + H), diabetes treated glibenclimade (D + G), Isoproterenol alone induced (I), diabetes induced isoproterenol (D + I), and diabetes induced isoproterenol treated hesperidin myocardial infarction (MI) rats (D + I + H).

	Normal (N)	Diabetic (D)	Diabetic + Hesperidin (D + H)	Diabetic + Glibenclamide (D + G)	Isoproterenol (I)	Diabetic + Isoproterenol (D + I)	Diabetic + Isoproterenol + Hesperidin (D + I + H)
0 Week	92.36 ± 7.49	435.65* ± 9.15 (371.6)	425.86* ± 11.54 (361.08)	438.89* ± 8.18 (375.19)	94.18ns ± 6.35 (1.97)	448.12* ± 9.15 (406.8)	459.67* ± 8.16 (397.6)
1stWeek	95.48 ± 6.23	428.26* ± 8.54 (348.5)	354.16# ± 9.62 (270.9)	382.65# ± 6.85 (300.7)	94.29ns ± 8.18 (−1.246)	445.16ns ± 7.85 (387.1)	406.89† ± 6.85 (326.1)
2nd Week	98.25ns ± 9.32	425.19* ± 7.19 (332.76)	308.27# ± 8.55 (213.7)	336.84# ± 8.66 (242.8)	99.15ns ± 9.23 (0.916)	445.43ns ± 8.16 (363.5)	367.65† ± 6.59 (274.1)
3rd Week	94.73 ± 8.35	426.38* ± 6.17 (350.1)	266.18# ± 7.97 (180.9)	282.68# ± 9.48 (198.4)	95.83ns ± 10.46 (1.16)	447.89 ns ± 6.47 (393.8)	289.65† ± 8.53 (205.7)
4th Week	93.14 ± 8.29	429.15* ± 8.14 (360.7)	235.28# ± 8.63 (152.6)	244.68# ± 8.47 (162.7)	95.18ns ± 9.18 (2.19)	449.18 ns ± 8.54 (403.7)	214.75† ± 9.47 (130.5)

Values are mean and ± S.D of six rats in each group, ns = non-significant.
Values in parentheses are the percent change from control.
*p < 0.01 as compared with normal rats; #p < 0.01 as compared with diabetic rats; †p < 0.01 as compared with Diabetic + Isoproterenol.

activity of lipid marker triglycerides in the blood where the effect was being decreased in hesperidin treated diabetic rats (12.48). A significant decrease in the blood cholesterol and TG concentrations was observed in hesperidin fed hypercholesterolemic rats but not in control hypercholesterolemic rats (24.07) (Table 4). The Hesperidin treatment significantly reduced the activity levels of HDL (−41.42), LDL (234.29), VLDL (24.02) and reduced total cholesterol levels in the blood of rats when compared to controls. The changes in lipid profile and antioxidant status in the blood stream of control and experimental groups were shown in (Table 4). Atherogenic index and the diabetic cardiac risk factor of hesperidin hypercholesterolemic rats were significantly lower than those of the hypercholesterolemic control rats (Table 4).

4. Discussion

Streptozotocin (STZ) is well known cytotoxic chemical for pancreatic islet beta-cells and it is extensively used to induce diabetes mellitus in animals. In the present study intraperitoneal administration of streptozotocin (STZ) (50 mg/kg body weight) to the normal rats effectively induced diabetes. The STZ damage the β-cells of the islets of Langerhans in pancreas causes the lower insulin production levels in diabetic rats, it lead to the increase the plasma glucose levels turns to diabetic. In hesperidin treated diabetic rats, the blood glucose levels were significantly decreased, it was accompanied by an increased plasma insulin levels by β-cells by the activation and reduced antioxidants levels in the blood through marinating the balanced insulin levels in diabetic rats.

The present investigation mainly on exposure of rats to STZ caused extensive changes in blood glucose levels of rat's turns to diabetic and isoproterenol induced diabetic rat's turns to DMI rats and treatment with hesperidin to revels the properties of hesperidin. The ingestion of hesperidin bioactivity was significantly effective in lowering the LDL and raising HDL concentrations in hypercholesterolemic rats. The beneficial effects of hesperidin were also reflected in the atherogenic index and the cardiac risk factor, both of which were lower in hesperidin treated hypercholesterolemic diabetic rats rather than in control rats. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase [12–14]. Recently Dokumacioglu et al. reported that statins, cholesterol-lowering agents, induce bone formation and inhibits the lipid profiles both *in vitro* and *in vivo* reported that hesperidin exerts protective effects against oxidative stress against the effects of increased serum glucose and lipid levels [15]. It is well known that 3-hydroxy-3-methyl- glutaryl-CoA (HMG-CoA) reductase inhibitory action on effective in lowering plasma cholesterol in most animal species, including humans, and these inhibitors are now widely used as hypocholesterolemic drugs [16], demonstrated that in rats, naringin and hesperidin significantly lowered the plasma and hepatic levels of cholesterol and TG by inhibiting HMG-CoA reductase and acyl-CoA: cholesterol acyltransferase (ACAT). Furthermore, hesperidin enhances expression of the gene encoding the LDL receptor [17], these are some possible mechanisms underlying the hypolipidemic effects of hesperidin; however, we did not measure the activities of HMG-CoA reductase and ACAT or the expression of the gene encoding the LDLr. Here we demonstrated increasing adiponectin, which can reduce lipid accumulation, by hesperidin administration. The cholesterol molecules travel through the blood stream in globular packages called as lipoproteins and there are different kinds depending on their size, density and stability. There are three major forms of cholesterol, such as HDL, LDL and VLDL. In the present study hesperidin exhibits a significant decreased in the HDL and lowered TC, LDL, total lipids and TG plasma levels in rats fed a cholesterol-containing diet [18]. The elevated serum cholesterol might reflect in the elevation of these cholesterol fractions during lipoprotein levels disrupt the levels of serum and cellular lipid and which may account for the genesis of DCVD and is associated with distinct

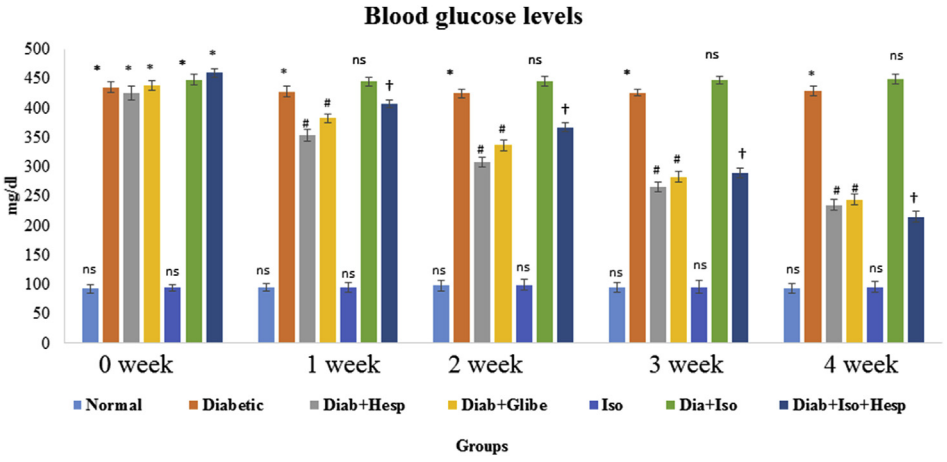

Fig. 3. Effect of hesperidin on blood glucose levels changes observed in 4 weeks in all experimental groups Normal, Diabetic, diabetic treated hesperidin (Diab + Hesp), diabetes treated glibenclimade (Diab + Glib), Isoproterenol (Iso), diabetes induced isoproterenol (Diab + Iso), and diabetes induced isoproterenol treated hesperidin myocardial infarction (MI) rats (Diab + Iso + Hesp).

pathological changes including dyslipidemia. It has been reported that alterations in the HDL may cause chronic heart diseases. Previous studies elucidate the mechanism involved in the cholesterol-lowering effect of dietary hesperidin have been reported English (2004) [19]. It has been suggested that hesperidin dietary phenols exhibits hypocholesterolemic effects might bind bile acids to reduce their entry into enterohepatic circulation, which then leads to an increase in gut bile acid secretion [20]. As a result, the liver responds by increasing hepatic conversion of cholesterol into bile acids, thus, reducing its circulating levels. In diabetes, hypertriglyceridemia and hypercholesterolemia are associated with the consequences of hyperinsulinemia, insulin resistance, and glucose intolerance [21]. In the current study, both diabetic and hypercholesterolemic disease, rats had significantly increased plasma TG concentration compared with normal non-disease rats. These elevated plasma TG concentrations were attenuated by oral supplementation of hesperidin. Although the activity of anti-hyper cholesterolemia of hesperidin was observed in hesperidin treated rats, it was not the case in diabetic rats. The reason for the difference in anti-hypercholesterolemic activity of hesperidin between the 2 disease models is not clearly known. However the inconsistent effects of hesperidin may be because of the different severity of diseases associated with the two models taken together, these present results are in agreement with those results reported in earlier studies with hesperidin [22]. The reduction observed in plasma LDL and elevated HDL concentrations were associated with lower liver weight with non-fatty condition as well as lower liver TC and TG concentrations in hypercholesterolemic rats fed high fat diet by treating with hesperidin. The observed beneficial effects of hesperidin on hypercholesterolemia are likely to be complex, probably involving a combination of bioactive glycosylated flavonoid components in the peel act as a lipid-lowering medication for elevated lipid (fat) levels of diabetic rats.

The elevated atherogenic effects of LDL have been attributed to oxidized lipid components such as esterified and unesterified peroxidized lipids, lysophosphatidylcholine, and cholesterol oxidation products [23,24]. The LDL oxidation process can lead to its subsequent aggregation, which further increases cellular cholesterol accumulation. Incubation of macrophages with native LDL (nLDL) does not lead to internalization of excess cholesterol due to down-regulation of the LDLr [25] on the other hand, oxLDL is recognized and internalized by macrophage scavenger receptors such as scavenger receptor-A (SR-A) [26] and SR-B (also known as SR-BI [27], which does lead to cholesterol accumulation [28]. Lysosomal cholesterol esterase then catalyze the generation of free cholesterol from lipoprotein-derived cholesteryl esters, and a proportion of the resulting free cholesterol is trafficked to intracellular membranes such as the endoplasmic reticulum (ER) membrane high levels of free cholesterol may affect the fluidity and permeability of membranes. Hesperidin added to the diet not only lowered serum and hepatic cholesterol, and the hesperidin, a citrus flavonoids, increased flow mediated dilation and reduced circulating inflammatory biomarkers, hs-CRP, serum amyloid A protein, soluble E selectin [29], decreased adhesion of monocytes, expression of vascular cell adhesion molecules-1 and generally improved vascular function with metabolic syndrome results. Citrus flavonoids showed the cardiovascular protection activity of decrease in total cholesterol with significant alterations in the HDL, LDL and VLDL in the plasma levels.

5. Conclusions

In conclusion, we observed that hesperidin normalizes blood glucose levels by altering the activity of glucose-regulating enzymes, and lowering serum lipid levels in STZ-induced diabetic MI rats without any body weight loss due to the modulatory effect in biotransformation enzymes. Hesperidin reports showed excellent bio-medicinal properties and works on by decreasing the production of excess cholesterol by the liver. Hesperidin supplementation showed many bio beneficial actions like decreased levels of inflammatory markers, increased cognitive functions and also some chemo preventive actions. There is no effects were observed on cardiovascular risk markers such as blood pressure, and artery stiffness etc. when taking as oral supplementations. Considering all these facts and our research results, hesperidin shows an antioxidant property which paving a way to consider bioactive compound to treat as biomedicine in both hypoglycemic and hypolipidemic actions against the STZ induced DMI rats.

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None.

CRediT authorship contribution statement

Somesula Swapna Rekha: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Jangampalli Adi Pradeepkiran:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Matcha Bhaskar:** Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Table 4
Effect of hesperidin on serum total cholesterol, triglycerides, HDL, LDL and VLDL in all experimental groups normal (N), STZ induced diabetic (D), diabetic treated hesperidin (D + H), diabetes treated glibenclimade (D + G), Isproterenol alone induced (I), diabetes induced isoproterenol (D + I), and diabetes induced isoproterenol treated hesperidin myocardial infarction (MI) rats (D + I + H).

	Normal (N)	Diabetic (D)	Diabetic + Hesperidin (D + H)	Diabetic + Glibenclamide (D + G)	Isoproterenol (I)	Diabetic + Isoproterenol (D + I)	Diabetic + Isoproterenol + Hesperidin (D + I + H)
Total cholesterol (m mol/l)	58.52 ± 3.27	117.36* ± 5.27 (100.55)	83.07# ± 5.13 (41.95)	75.16# ± 3.75 (28.43)	125.16* ± 4.68 (113.87)	155.72# ± 4.65 (166.09)	98.64† ± 3.18 (68.56)
Triglycerides (m mol/l)	74.35 ± 2.14	116.15* ± 4.18 (56.22)	92.25# ± 3.65 (24.07)	83.63# ± 3.43 (12.48)	112.14* ± 5.17 (50.83)	136.64ns ± 4.25 (83.78)	96.45† ± 5.13 (29.72)
HDL (m mol/l)	31.87 ± 2.16	13.15* ± 2.07 (−58.74)	18.67# ± 3.15 (−41.42)	14.15ns ± 4.15 (−55.60)	15.67* ± 3.45 (−50.83)	11.34ns ± 1.86 (−64.42)	16.34ns ± 2.56 (−48.73)
LDL (m mol/l)	15.75 ± 2.64	84.35* ± 4.05 (435.56)	52.65# ± 1.84 (234.29)	62.38# ± 3.65 (296.06)	86.24* ± 3.26 (447.56)	92.17# ± 2.38 (485.21)	59.17† ± 3.83 (275.68)
VLDL (m mol/l)	33.81 ± 2.86	52.82* ± 3.87 (56.23)	41.93# ± 2.54 (24.02)	33.01# ± 3.71 (−2.37)	50.97* ± 3.74 (50.75)	62.11# ± 4.58 (83.70)	43.84† ± 4.63 (29.67)

Values are mean ± S.D of six rats in each group, ns = non-significant.
Values in parentheses are the percent change of control.
*$p < 0.01$ as compared with normal rats; #$p < 0.01$ as compared with diabetic rats; †$p < 0.01$ as compared with Diabetic + Isoproterenol.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnim.2018.12.004.

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