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High Fat Diet Mediated Inflammatory Molecules Induced Development OF Diabetogensis In Rats Ameliorated by Hesperidin

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Abstract

High fat diet rich in saturated fatty acids caused serious health problems. Citrus fruits rich in flavonoids as Hesperidin that exerts different biological activities as anticancer. The wastes produced during food processing yield a huge amount of citrus residue, these wastes are a good source of hesperidin. This study investigated the complications of high fat diet (HFD) in development of metabolic syndrome as insulin resistance and evaluated the role of hesperidin to protect against these abnormalities. To achieve this purpose , four groups of rats were included, Gr I: Control; Gr II: rats treated with 30 mg hesperidin /kg bw/day; Gr III: HFD rats fed on diet contain 45 % lard fat and Gr IV:HFD treated with 30 mg hesperidin /kg bw/day. After 3 months, fasting sugar, HA1c, insulin, HOMA-IR, lipid profile, malondialdehyde, antioxidant activities, TNF-α and IL-1β were evaluated. Data obtained showed that, rats fed HFD diet showed a significant elevation of the levels of HA1c, MDA, LDL-c, TG, TNF-α and IL-1β while antioxidant enzymes activities were significantly decreased in rats fed HF versus control (p<0.001). Hesperidin improve HOMA-IR, decreased LDL-c, TG, increased antioxidant activity and decreased MDA. The levels of TNF-α and IL-1β were significantly lowered in rats treated with hesperidin versus untreated group (p<0.001). In conclusion, hesperidin ameliorated abnormalities induced by HFD diet as indicated by insulin sensitivity, enhanced antioxidant activity and reduced inflammatory mediators release.

Keywords: High fat- -toxicity- insulin resistance- inflammatory mediators- rats.

1.Introduction

Insulin resistance (IR) is found to be the most factor implicated in the development of metabolic diseases, CHD and diabetes [1]. The fundamental aspect in the etiologic of these disorders is linked to other complications including hyperlipidaemia [2]. This because, the saturated fatty acids interfere with insulin receptor and action [3] . The harmful effect of ROS lead to obesity-related pathologies 4] . The relationship between IR and diminished mitochondrial function still unclear, [4] . Hesperidin is one of flavonoides compounds that was isolated from the citrus fruits and wastes and exert different biological effects [5]. It exerts anti-inflammatory, antihypertensive and anticancer against different cancer cell lines. It was reported that, the beneficial effects of hesperidin used in management of many diseases as CVD, diabetes, obesity, asthma, aging [6]. In previous study, it was found that, hesperidin supplementation exerts a significant reduction on inflammatory mediators as CRP, IL-4, and IL-6 levels and served as anti-inflammatory [7]. Also, it exerts beneficial effect on bone homeostasis as it stimulate bone calcification and osteoblast [8]. Hesperidin found to be important in prevention of some age-related dissease. It also it suppresses the melanoma cell line growth [8-10]. The rational of current study evaluated the role of hesperidin against complications induced by HFD as insulin resistance and dyslipidemia in rats. To achieve this purpose, the antioxidant, anti-inflammatory and HOMA-IR were evaluated.

2. Materials and Methods.

2.1Animals.

The handing of animals was carried out according to ethical committee of Ain Shams University. Four groups of male *Wister* albino rats weighing about 100 gm (8 rats for each group) were used in this study. Hesperidin was purchased from Sigma Company with purity (99.5%) and was dissolved in DMSO. Group I**:** Control group, rats fed standard normal diet for 3 months. Group II: Rats maintained on normal diet were received oral dose of 30 mg/kg b.w of hesperidin for 3 months. Group III (HF diet): Rats were fed on HFD containing 45 % lard fat, 15 % protein, 30 % carbohydrate, 4 % fibre. Group IV: Rats fed on HFD and received oral doses of 30 mg/kg bw of hesperidin. At the end of 3 months, animals were fasted for 14 hours; water was available only. Blood was collected by decapitation after anaesthesia by thiopental. Blood was collected for separation of serum and plasma.

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2.2. Biochemical investigations

2.2.1.Assay of insulin resistance (HOMA-IR) and diabetic markers.

Serum from all animals were subjected for measurement of fasting glucose, insulin, Glycated haemoglobin (HA1c) by commercially available kits. HOMA-IR was calculated as following:
HOMA-IR = [Fasting insulin [pmol/l]) x (Fasting blood glucose [mmol/l])/22.5. Lipid profile levels including t.cholesterol, TG, LDL-c and HDL-c were evaluated by kits from Biomed.

2.2.2.Determination of IL-1β and TNF-α in MDA, SOD, and GPX.

The concentrations of IL- 1β and TNFα were determined in serum by ELISA Kits from BIOMED while MDA level , the activity of SOD and GPX were determined by colorimetric kits

2.2.3.Statistical analysis.

Data was expressed as mean with standard deviation (SD). Differences between groups were analysed by unpaired t test. P values of < 0.05 was considered significant.

3. Results.

Data obtained showed that, the levels of fasting glucose, HA1c as shown in table (1) were significantly elevated in rats fed on HFD versus untreated ($p < 0.001$ for each). In addition, a significant elevation in insulin level and HOMA-IR values in rats fed HF diet compared with normal rats. On the other hand , rats treated with hesperidin showed a significant reduction fasting glucose, HA1c , insulin level and HOMA-IR ($p < 0.001$) versus untreated. HOMA-IR was improved but not returned to normal values. Lipid profile in (table 2) revealed a significant elevation in levels of serum total cholesterol, triglycerides, LDL-c and a significant decreased in HDL-c in rats fed on HFD compared with normal rats ($p<0.001$). Rats fed HFD treated with hesperidin showed improvement in total cholesterol, LDL-c and HDL-c but not in triglycerides. Oxidative damage makers were presented in table (3), it was found that, rats fed HFD showed a significant increase in serum MDA level versus normal. However, hesperidin treated rats decreased its level compared with untreated ($p<0.001$). There was a significant reduction in glutathione reductase and glutathione peroxidase ($p < 0.001$) in rats fed HFD compared with control rats. Rats treated with hesperidin improved oxidative stress by reduction of MDA and increased antioxidant enzymes.
Data in Figs 1 showed that, in rats fed on high fat a statistical increase in IL-1β, TNFα levels in serum of rats fed HFD versus control ($p<0.001$). Treatment with hesperidin reduced their levels ($p<0.001$) versus untreated rats. It was observed that, Hesperidin in normal rats did not significantly caused changes in the above parameters.

Table (1): Percent Changes in body weight increase, levels of fasting blood glucose and HbA1c in all studied groups (Mean ±SD)

Parameters	GP 1	GP II	GP III	GP IV
FBS (mg/dl)	74± 2.87	81±2.4	190±16 [a,b]	162±13.9[b]
HA1c (%)	4.1± 0.41	4.3±0.11[a]	6,8±0.63 [b]	5.7±0.78 [b]
Insulin (pmol/l)	320±16	330±16	531±46	390±24
HOMA-IR	2.1± 0.21	2.2± 0.2	4.5± 0.61	3.3± 0.11

(a): significant versus control. (b) significant versus untreated with hesperidin

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Table (2): Serum levels of lipid profile (T.cholesterol,triglyceride, LDL-c and HDL-c) in all studied groups (Mean ±SD).

Parameters	GP 1	GP II	GP III	GP IV
T.cholesterol (mg/dl)	81±4.3	87±5.7	131±10.1[a]	115±9.9 [a]
Triglyceride (mg/dl)	90±7.1	87±7.3	160±16	139±14
LDL-C(mg/dl)	27± 1.3	30±1.9	41±3.4 [a,b]	39±2.1[b]
HDL-c(mg/dl)	34± 2.1	37±3.2	31±0.13 [b]	30±3.2 [b]

(a): significant versus control. (b) significant versus untreated with hesperidin .

Table (3): Serum oxidative stress markers (MDA, SOD, glutathione peroxidase and glutathione reductase) in the studied groups (Mean ± S.D)

Groups	MDA (µmol/mg protein)	SOD (µg /mg protein/g tissue)	Glutathione peroxidase (U/mg protein)	Glutathione reductse (U/mg protein)
Group I	66.7 ± 28	45 ±2.1	987 +90	2112 +154
Group II	72 ± 4.7	47 ±1.3	1110 ±95	2200±172
Group III	110 ± 9.2 [a,b]	79 ±4.4 [a,b]	780 ±84 [a,b]	1179 ±143 [a,b]
Group IV	105± 5.5 [a,b]	87±6.4 [a,b]	860 ±86 [a,b]	1540±153 [a,b]

(a): significant versus control. (b) significant versus diabetic.

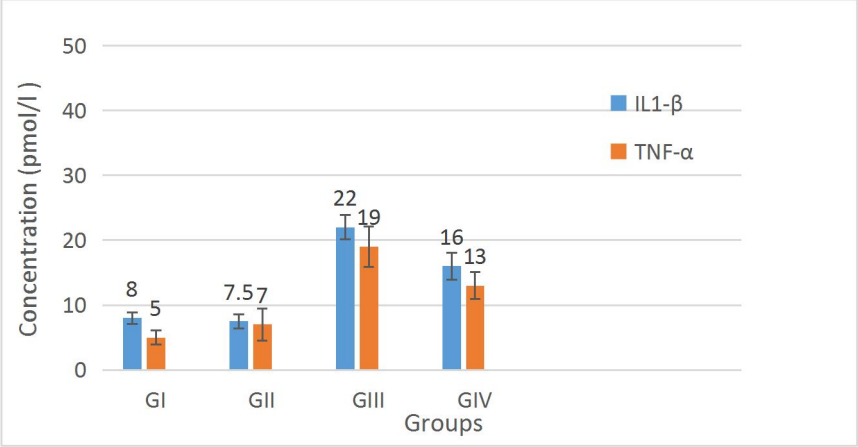

Fig. 1. The level of IL-1β and TNF-α in all studied groups (Mean ±SD).

4. Discussion

High fat rich diet with sedentary life style can increased incidence of cardiovascular diseases as atherosclerosis, insulin resistance and diabetes. Dyslipidemia associated with elevated LDL-c exposed to oxygen species can oxidized LDL-c and increased incidence of atherosclerosis.

Hesperidin is a flavonoid compound that exert different functions. In this study, It was reported that, hesperidin given to diabetic rats reduced blood glucose and HA1c [11]. Also, hesperidin lowered blood glucose in hyperglycemic rats [12]. Data obtained showed that, hesperidin treatment in rats fed HF diet decreased levels of FBS, HA1c and versus untreated rat. The effect of hesperidin may be due to decreased glucose absorption, reduced polyol pathway and scavenger for glucose to inhibit glycation of hemoglobin

We try to discuss the mechanism that associate the related to insulin resistance (high HOMA-IR values) in rats fed HF diet. This is may be due to blocking of insulin receptors by saturated fatty acid for masking binding to insulin. Other explanation, increased free radicals and oxidation of LDL-c to form ox-LDL-c that form foam cell with macrophage and decreased action of insulin. This is reversed by hesperidin treatment. This is may explained by protection of pancreatic beta cells against ROS and damage. It was reported that, hesperidin, enhanced antioxidant activity after eating citrus food [13]. Also, if given orally, it stimulate overall antioxidant capacity and reduces ROS that caused damage to fat, protein and DNA. Thus hesperidin act as free radical scavenging activity [14]. It suppress release of IL-6, TNF, and iNOS [15]. In addition, hesperidin modulate prostaglandin synthesis and COX-2 expression pathway [16]. It has an antiinflammatory effect and increased antioxidant abilities.

In our study, administration of hesperidin lead to increased antioxidant efficacy. Oxidative stress contributes in cellular abnormalities and development of many diseases. One of the major diseases is diabetes due to free radicals decreased antioxidant activity [14]. In current study, rats fed HFD increased free radicals and decreased antioxidant activity. Hesperidin found to enhance antioxidant activity and decreased conjugated diene, via scavenging free radicals and upregulate antioxidant enzymes activity. This is in accordance to study of [15] who reported that, diabetic rats given hesperidin exerted anti-oxidative agent and explained its role as dietary supplement containing hesperidin protecting against diabetic complications. Rats fed HFD showed significant elevation in serum triglycerides, LDL-c, t.cholesterol and reduced HDL-c. It was found that, HFD increased release of inflammatory mediators (IL1-β and TNF) compared with normal diet. Hesperidin given to rats HFD showed anti-inflammatory effect by lowering their levels versus untreated. The protective effect of hesperidin due to high potent antioxidant effect and decreased of lipid peroxidation of normal rats.
This is in accordance with [16-20] who reported that, hesperidin improves diabetic rats induced by STZ. In addition, hesperidin (5-20 mg/kg bw) treated diabetic rat modulated oxidative stress by potentiate antioxidant activity and decreased lipid peroxidation. The effect was observed through increased the activities of SOD and GPxs and reduced MDA level in target tissue [21].
The protective effect of hesperidin was explained by, protect cell membrane from peroxidation, and cell integrity, decreased release of inflammatory mediators [22,23].

5.Conclusion

In conclusion, consumption of hesperidin rich foods may protect against incidence of diabetes and CVD , its action was mediated through suppression the release of inflammatory mediators, improving antioxidant capacity, enhance insulin sensitivity.

6. Funding: Not applicable.

7. Conflict of interest: The author declare there is no conflict of interest.

List of abbreviations: Glycated haemoglobin (HA1c), cardiovascular disease (CVD), Insulin resistance (HOMA-IR), LDL-c (low density lipoprotein), HDL-c (high density lipoprotein), HFD (high fat diet), SOD (superoxide dismutase) and GPxs(glutathione peroxidase) , MDA(malondialdhyde).

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