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Impact of Nigella Sativa, Omega-3 Fatty Acids and Chromium Picolinate on NF-κB /leptin-insulin Axis in Obese Subjects with Non-alcoholic Fatty Liver Disease

Noha M. Shafik[1], Reham A. Mariah[1,4], Hussam Baghdadi[4], Sherine Ragab Shafik[2], Loai Mohammed El-Ahwal[3], Salah Mohamed El Sayed[4,5,*], Nassar Ayoub Abdellatif Omar[6]

[1]Departments of Medical Biochemistry, Faculty of Medicine, Tanta University, Egypt
[2]Ph.D in Medical Biochemistry, Faculty of Medicine, Tanta University, Egypt
[3]Department of Internal Medicine, Faculty of Medicine, Tanta University, Egypt
[4]Department of Clinical Biochemistry and Molecular Medicine, Taibah Faculty of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia
[5]Department of Medical Biochemistry, Sohag Faculty of Medicine, Sohag University, Egypt
[6]Department of Anatomy, Faculty of Medicine, Sohag University, Egypt
*Corresponding author: salahfazara@yahoo.com, drsalahpediatr@yahoo.com

Abstract **Background:** The global problem of obesity epidemic involves an increased risk of non-alcoholic fatty liver disease (NAFLD) whereby oxidative stress induces fibrogenesis. **Objectives:** to assess the coordinated impact of obesity management strategy on nuclear factor kappa beta/p65 mRNA expression and biochemical parameters of oxidative stress, glycemic status and adipokines. **Subjects and methods**: This study was carried out on 60 subjects classified into, 15 normal healthy controls (group I) and 45 obese subjects who were divided equally into three groups: obese subjects with NAFLD (group II), obese subjects who received natural product supplements (nigella sativa, green tea andchromium picolinate) (groupIII),obese subjects who received omega-3 fatty acids (group IV) andthose who received caloric restricted diet (CRD) and exercise for 6 months. All groups were subjected to measurement of body mass index, waist to hip ratio (WHR), spectrophotometric measurement of serum levels of glucose, malondialdehyde (MDA) and total antioxidant capacity (AOC%), NF-κB /P65 subunit expression levels by real time-PCR in peripheral bloodmononuclear cells, estimation of levels of insulin, insulin like growth factor-1, leptin, adiponectin, fibronectin and oxidized LDL by enzyme linked immunosorbent assays. **Results:** There was a significant decrease inNF-κB/p65 mRNA expression in peripheral blood mononuclear cells, reduction in the levels of oxLDL, decreased insulin resistance and decreased leptin resistance which might be linked to hypoadiponectinemia. Levels of (AOC %) were significantly elevated after treatment. This was evident alongside reduction of BMI, WHR and fibrogenic potential in NAFLD. **Conclusion:** natural product supplements, CRD and exercise ameliorated the fibrogenic and atherogenic consequences of immune-inflammatory and oxidative stress-induced pathological mechanisms associated with obesity.

Keywords: *non-alcoholic fatty liver disease, NF-κB/p65, nigella sativa, chromiumpicolinate, omega-3 fatty acids*

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1. Introduction

The observation of increased generation of free radicals with high caloric diet is interlinked to atherogenic and fibrogenic liability [1] alongside the observed rapidly increasing rates of overweight and obesity- induced pathological mechanisms [2]. They are determined by genetic and transcriptional factors influencing the body mass index (BMI) and associated with simple steatohepatitis and non-alcoholic fatty liver disease (NAFLD) which represents an emerging worldwide concern [3]. Hence, body weight and adiposity can increase over life-time, especially with hyperleptinemia and insulin resistance in obesity prone individuals with a genetically raised threshold for sensing a variety of hormonal and metabolic signals [4]. A key role in the cross- talk among hepatic stellate cells (HSCs) and liver macrophages correlated with fibrogenesis involve the immune-inflammatory signaling nuclear factor kappa B (NF-κB) [5] and growth factor interactions (insulin-like growth factor-1) alongside the role of adipokines (leptin and adiponectin) [6]. Moreover, the involvement of oxidative stress in obesity induced NAFLD activates hepatic stellate cells and macrophages [1] and associated

with derangement of lipid homeostasis, insulin resistance, immune tolerance response and pericentral fibrogenesis [5]. In contemplation, such inflammatory links involvingNF-κB which exist between hyperinsulinemia via increased IGF-1 and insulin resistance [7,8] The induction of pro-inflammatory stimuli involving nuclear factor kappa beta (NF-κB) alongside insulin resistance (IR) influences adipocytokines balance that affects satiety and fibrogenesis implicating role of fibronectin increments [9]. It coordinates with reactive oxygen species (ROS) modulation of the redox state in the liver via NF-κB transcription factors that activates hepatic stellate cells [10]. Due to its strong transcriptional activity, the p65 subunit of NF- κβ (NF-κB/p65) may be responsible for most of NF-κB's transcriptional activity [11].

2. Subject and Methods

The study included a total of 60 cases that were selected from Tanta University Hospital outpatient clinic of tropical medicine, within the age range from 33 to 45 years. All cases under study were certified to be free of viral, protozoal and parasitic infection. They were classified into 4 groups.

Group I: Control group included 15 healthy non-obese subjects.

The categorization of these non-obese cases was based on anthropometric indices (12), body mass index (BMI) = weight in Kg / height in m^2 and waist to hip ratio (WHR) = waist girth / highest hip girth (13).

Obesity was expressed as BMI > 25 and < 35 Kg / m^2 and WHR > 0.8. In addition, the selected obese subjects were identified by HOMA-IR to exhibit aspects of insulin resistance and were classified into three groups (from group II to group IV) of 15 subjects in each group as follows.

Group II: included 15 obese cases with sedentary life style.

Group III: included 15 obese cases undergoing caloric restricted diet, daily walking forhalf an hour, daily intake of nigella sativa and chromium picolinate supplementationas well as drinking 3 cups/ day of green tea beverage regularly.

Group IV: included 15 obese cases undergoing caloric restricted diet, daily walking forhalf an hour, daily intake of nigella sativa, omega-3 fatty acids and chromium picolinate supplementation as well as drinking 3 cups/day of green tea beverage regularly. All subjects were evaluated clinically. Also, abdominal ultrasonography examination was performed to verify the existence of NAFLD.

Informed written consent was obtained from all participants. The study protocol was approved by the Local Research Ethics Committee, Tanta University and was in accordance with the principles of the Declaration of Helsinki II.

Exclusion criteria: Cases with a history of essential hypertension, renal disease,

thyroid problems or diabetes mellitus.

Biochemical assay: Venous blood samples (10 ml) were collected by venipuncture between 8 and 10 a.m. after an overnight fasting and divided into 2 volumes: one, allowed to clot (serum) and the second was transferred into vacutainer tubes with 5% EDTA (plasma). Both volumes were centrifuged at 3,000 rpm for 5 minutes then, stored at – 20 c until used for the assays of:

1. Glucose level by spectrophotometric quantitation (Diamond diagnostics, Germany) using oxidase method [14].
2. The validation of insulin by Enzyme Linked Immunosorbent Assay (ELISA) using ELISA kit (Immunospec corporation catalog No. E 29-072, USA) [15]
3. The insulin resistance was identified using the homeostasis model assessment of insulin resistance (HOMA-IR) by the following formula:
   HOMA-IR (mmol/ L ×μ IU/ ml) = fasting blood glucose (mmol/L) × fasting insulin (μ IU/ ml) / 22.5 according to Matthews et al., 1985 [16].
4. The evaluation of Leptin in human plasma using Max human leptin Assay ELISA kit supplied by Assay Pro [17].
5. The assessement of adiponectin levels using ELISA kits for the Assay Max Human Adiponectin by the aid of catalog No. EA-2500-1, USA [19].
6. Plasma fibronectin was determined by ELISA method, the kit supplied by Cloud Clone-Corp [20].
7. Oxidized low- density lipoprotein (ox LDL) was determined by ELISA kit provided by Cell Biolabs Inc [21]
8. Total anti-oxidant capacity (AOC %) was analyzed by the phosphomolybdenum method [22]. The serum to be analyzed is treated with 5% TCA to precipitate the proteins. The deproteinised sample is then treated with the total antioxidant capacity (TAC) reagent, which contains 28mM sodium dihydrogen phosphate and 4mM ammonium heptamolybdate, in a 0.6M of Conc. H2SO4, at 90oC for, 90 minutes in a water bath. Following incubation, the mixture is cooled and the optical density of the green complex which is formed is read at 695nm by spectrophotometer.
9. Malondialdehyde (lipid peroxidation product) was measured usingthe thiobarbituric acid reactive substance (TBARS) as the method described by Ohkawa et al., 1979 [23].

Preparation of peripheral blood mononuclear cells (PBMCs): PMNCs were prepared by density gradient centrifugation using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Heparinized blood was carefully layered on Ficoll, and PBMCs were harvested from the white interphase after centrifugation for 30 minutes at 400 g, at room temperature and washed with phosphate buffered saline (PBS) (24). The PBMCs samples were stored at −80◦C till the samples were further processed for RNA isolation.

Real-time PCR: Total RNA from PBMCs was prepared using Trizol reagent (Gibco BRL Life Technologies) according to the manufacturer's instructions. The integrity of total RNA was checked by electrophoresis through 1% agarose gel. RNA samples were then stored at -80°C. cDNA synthesis was performed using the RevertAid H Minus First Strand cDNA Synthesis kit (#K1632,Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions. Real-time PCR was carried out with single stranded cDNAs. PCR reactions were performed using Power SYBR Green PCR Master Mix and 7500 Fast Real-Time PCR System (Applied

Biosystems, CA, USA). Primer sequences specific for the NFκB /P65 (No: NM_021975.3) according to Sun et al., 2012 [25] are: Fwd: 5′-ATCTGCCGAGTGAACCGAA ACT-3′; and Rev: 5′-CCAGCCTGGTCCCGTGAAA-3′ ; β-actin (No:NM_001101.3): Fwd: 5′- TGGCATTGC CGACAGGATGCAGAA-3, Rev:5′-CTCGTCATACTC CTGCTTGCTGAT -3′. β-actin primers were used as an internal control. Real-Time PCR was carried out, in duplicate, by 40 cycles of 95°C for 10 sec and 60°C for 1 min. Comparative Ct (threshold cycle) method was used to determine the relative amounts of the products, according to the Applied Biosystems instructions. Conventional PCR was performed with the DreamTaq polymerase (#EP0701, Thermo Scientific Fermentas, St. Leon-Ro, Germany). All expression data were normalized by dividing the target amount by the amount of β-actin used as internal control for each sample

Statistical Analysis: Analysis were performed using the SPSS software version 20.0 (SPSS Inc, Chicago, IL). Baseline characteristics are presented as mean ±standard deviation for the studied parameters. Comparisons between groups were conducted using ANOVA test followed by Tukey's post-hoc test for multiple comparisons. The correlations between NFκB/p65 and thestudied biochemical parameters were calculated using Pearson's correlation coefficient.

3. Results

(Table 1) The effect of obesity management strategy on BMI, WHR and levels of insulin, insulin growth factor-1, leptin and adiponectin in obese individuals:

There is a significant elevation of body mass index and waist to hip ratio in obese individuals (group II**)** (p <0.001*) as compared with the control group (group I). Also, the levels of insulin, insulin like growth factor-1, HOMA-IR, leptin were significantly elevated in obese individuals (group II**)** (p <0.001*) compared with the control group (group I). On the other hand, adiponectin levels were significantly lower in obese individuals (group II**)** (p <0.001*) compared with the control group (group I). Obesity management strategy significantly decreased BMI, WHR, HOMA-IR and the levels insulin, leptin in (group III > IV). Also, the levels of adiponectin were significantly elevated in group (III >IV) after treatment of obesity.

Table 1. Effect of obesity management strategy on BMI, WHR and levels of insulin, insulin growth factor-1, leptin and adiponectin in obese individuals

	Groups				ANOVA	
	Non-obese control subjects (group I) (n=15)	Obese subjects with fibrogenic Liability (group II) (n=15)	Obese subjects receiving NS & Cr (group III) (n=15)	Obese subjects receiving NS, Cr & omega 3 plus (group IV) (n=15)	F	P-value
BMI (Kg/m2)	21.435±2.435	38.488±3.094 [a]	33.242±3.115 [ab]	28.928±1.480 [abc]	113.873	<0.001*
WHR	0.607±0.025	0.920±0.043 [a]	0.828±0.045 [ab]	0.764±0.025 [abc]	205.253	<0.001*
Insulin (µIU/ml)	3.985±1.306	15.475±5.663 [a]	9.476±2.654 [ab]	8.279±2.853 [ab]	13.474	<0.001*
Insulin growth factor-1(ng/ml)	160.460±19.810	240.737±22.833 [a]	196.146±30.673 [ab]	168.944±15.380 [bc]	33.862	<0.001*
HOMA-IR	1.4±0.54	9.9±7.2 [a]	5.56±1.2 [ab]	3.65±2.1 [abc]	26.227	<0.001*
Leptin(ng/ml)	17.421±2.410	40.458±4.188 [a]	31.276±2.636 [ab]	24.189±2.026 [abc]	169.693	<0.001*
Adiponectin (ng/ml)	19.407±2.074	12.793±3.454 [a]	17.869±3.119 [b]	22.819±4.940 [bc]	22.224	<0.001*

BMI: Body mass index, WHR: waist to hip ratio, **NG**: Nigella Sativa oil 450 mg (component in Baraka capsules). **Cr**: Chromium picolinate 200 mcg Equivalent to chromium 24.85 mcg component in chromium capsules).**HOMA-IR:**homeostasis model assessment of insulin**.**
Values are expressed as mean ± S.D for 60 subjects.
*****Statistically significant values at (*p< 0.001).
a= significant differences with group I
b= significant differences with group II
c= significant differences with group III

Table 2. Effect of obesity management strategy on fibronectin, total antioxidant capacity (AOC%), malondialdehyde (MDA) and oxidized LDL in obese individuals

	Groups				ANOVA	
	Non-obese control subjects (group I) (n=15)	Obese subjects with fibrogenic Liability (group II) (n=15)	Obese subjects receiving NS & Cr (group III) (n=15)	Obese subjects receiving NS, Cr & omega 3 plus (group IV) (n=15)	F	P-value
Fibronectin(ng/ml)	276.748±50.312	486.360±94.669 [a]	393.987±83.741 [ab]	324.348±66.984 [b]	21.654	<0.001*
AOC %	78.924±11.864	64.177±8.838 [a]	75.057±23.432 [b]	86.378±18.624 [abc]	4.904	0.004*
Lipid peroxidation product (MDA) (nmol/ml)	5.660±1.715	9.626±2.546 [a]	7.176±2.760 [b]	5.521±2.662 [b]	5.304	0.003*
Ox-LDL(ng/ml)	80.828±9.405	179.380±43.749 [a]	149.906±23.296 [ab]	113.695±16.463 [abc]	39.165	<0.001*

AOC %: Percent of Total Antioxidant Capacity**. MDA**: Malondialdehyde
Values are expressed as mean ± S.D for 60 subjects. **Ox-LDL**: Plasma oxidized LDL.
*****Statistically significant values at (*p< 0.05), (*p< 0.01) and (*p< 0.001).
a= significant differences with group I
b= significant differences with group II
c= significant differences with group III.

The levels of fibronectin, (MDA) and ox LDL were significantly elevated in obese individuals (group II) (p <0.001*) compared with the control group (group I). On the other hand, total antioxidant capacity (AOC %) levels were significantly lower in obese individuals (group II) (p <0.001*) compared with the control group (group I). Obesity management strategy significantly decreased the levels of fibronectin, (MDA). Also, ox LDL levels were significantly decreased in group (III >IV) after treatment of obesity. While, the levels of (AOC%) were significantly elevated in group (III and IV) after treatment of obesity.

(Table 3 and Figure 1) The effect of obesity management strategy on nuclear factor kappa beta mRNA relative expression (NF-κB/p65 mRNA/GAPDH ratio):

NF-κB/p65 mRNA expression were significantly elevated in obese individuals (group II) (p <0.001*) compared with the control group (group I). While after obesity management strategy in group (group III) and group (IV), the NF-κB/p65 mRNA was significantly decreased.

Table 3. Effect of obesity management strategy on NF-κB/p65 mRNA relative expression in obese individuals

	NFKB/p65 mRNA/GAPDH ratio					ANOVA	
	Range			Mean	± SD	F	P-value
Group I	0.012	-	0.029	0.021	± 0.004	2947.416	<0.001*
Group II	0.175	-	0.189	0.181	± 0.004		
Group III	0.093	-	0.116	0.103	± 0.006		
Group IV	0.070	-	0.083	0.075	± 0.004		

Tukey's test					
I&II	I&III	I&IV	II&III	II&IV	III&IV
<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Significant *p-value<0.001.

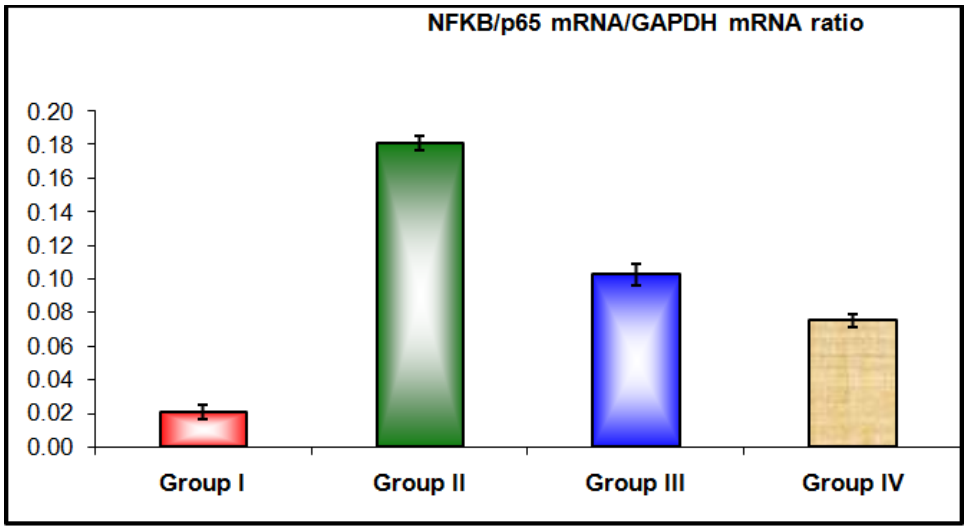

Figure 1. Effect of obesity management strategy on nuclear factor kappa B mRNA relative expression

(Table 4) Correlation analysis between the different studied parameters and NFκB/p65 expression in studied groups:

Using Pearson correlation test (Table 4), NF-κB/p65 mRNA expression and BMI, WHR showed significant positive correlations. Also NF-κB/p65 mRNA expression showed significant positive correlations with the levels of insulin, insulin growth factor-1, leptin,fibronectin, malondialdehyde (MDA) and oxidized LDL. On the other hand, significant negative correlations were detected between NF-κB/p65 mRNA expression and the levels of adiponectin and (AOC%).

Table 4. Correlation analysis between the different studied parameters and NFκB/p65 expression among obesity studied group (group II)

Correlations	NFKB/p65 mRNA	
	r	P-value
BMI (Kg/m2)	0.898	<0.001*
WHR	0.922	<0.001*
Insulin (µIU/ml)	0.767	<0.001*
Insulin growth factor-1(ng/ml)	0.792	<0.001*
Leptin(ng/ml)	0.938	<0.001*
Adiponectin (ng/ml)	-0.625	<0.001*
AOC %	-0.356	0.005*
Lipid peroxidation product (MDA) (nmol/ml)	0.427	<0.001*
Ox-LDL(ng/ml)	0.808	<0.001*
Fibronectin (µg/ml)	0.729	<0.001*

r= Pearson's correlation coefficient, *Significant at p <0.001* and <0.01*.

4. Discussion

The global problem of obesity epidemic may involve an increased risk of NAFLDwhereby oxidative stress induces fibrogenesis [26]. In consistence, obesity-associated oxidative stress is reflected by the assessed reduction of total antioxidant capacity (AOC %) in (groupII) (Table 2). This verified a lower defense to oxidative modification by reactive nitrogen species (RNS) and reactive oxygen species (ROS) in agreement to previous reports [1]. Concordantly, the associated increments of ox-LDL in group II (Table 2) in parallel to the increased values of lipid peroxidation product MDA is related to fibrogenic susceptibility confirmed by assessed increments of extracellular matrix protein, fibronectin, which is in agreement to previous reports [27]. Furthermore, the ox-LDL dual impact on atherogenesis and fibrogenesis reflected their coordination with proinflammatory cytokine increments of NF-κB/p65 mRNA expression which is a redox-sensitive proinflammatory transcriptional factor with fibrogenic potential in group II (Table 3). This was relevant to the deposition and accumulation of oxidized phospholipids, lipid peroxides, lysophospholipids and oxysterols in the arterial walls of obese subjects as noted elsewhere [28]. Moreover, increments of HOMA-IR and NF-κB/ p65 mRNA expression with ox-LDL agreed with the conjoint influence of glycemic status on inflammatory signals and lipid oxidation. The impact of NF-κB/ p65 mRNA expression was provoked by the role of obesity-associated insulin resistance promoting atherogenic and fibrogenic liability (Table 3). Also, Table 4 showedpositive correlations between NF-κB/ p65 mRNA relative expression and BMI, WHR, levels of, insulin, insulin like growth factor-1, oxLDL, fibronectin, leptin and malonialdehyde. While negative correlations were found between NF-κB/ p65 mRNA relative expression and levels of adiponectin and total anti-oxidant capacity. These findings ensure the inflammatory impact of obesity on NF-κB/insulin-leptin axis.

The amalgamated response to exercise, caloric restricted diet and supplements of nigella sativa, omega-3 fatty acids and chromium was promoted in group IV more than in group III and was associated with reduction in BMI and WHR together with lowering NF-κB/ p65 mRNA expression. An associated increase in assessed decrements oftotal antioxidant capacity (AOC %) might be involved in improvement of obese subjects with NAFLD (in group IV more than in group III).This was paralleled with reduction of oxLDL and MDA and counteracting the activation of NF-κB. It was verified by reduction of fibronectin, which was monitored in parallel to reduction of BMI and WHR following six months management strategy. The noted response was verified by an interlink between adjustment of hypoadiponectinemia and hyperleptinemia impact on metabolic signaling pathways and insulin resistance [29]. It was furthermore displayed by the coordinated impact of the assessed reduction of the adipocyte derived cytokine, adiponectin, which implements inefficiency in its ultimate exertion of its anti-inflammatory, anti-fibrogenic and anti-atherogenic effects particularly in endothelial cells and macrophages [30]. Furthermore, the outcome of management strategy was reflected by counteracting the assessed reduction of adiponectin, AOC% versus decreasing hyperleptinemia, hyperinsulinemia, oxLDL and malonialdehyde increments.

This was established by readjusting the deranged network of metabolic sensing neurons via normalizing the raised threshold in sensitivity of satiety centers of obesity prone individuals implementing their minimized response to the impact of inhibitory signals, which inform the brain when there is an excess of energy stores [31].

Based on that, the adapted CRD regimen conjoint with impact of exercise and natural product supplements may prevent the anabolic tilt in the central neuropeptide pathways with subsequent lowering of insulin and leptin levels for counteracting adiposity [32]. It reflects reduced adipogenesis via signaling pathways co-linking adipose tissue and hypothalamic centers for energy homeostasis [33] potentiating the lowering of BMI and WHR (monitored in group III and IV). It occurred via readjustment of strong influence of obesity on a series of peripheral tissues implying the role of the complex leptin-insulin axis [34]. In consequence, group III and IV had expressed rebalance of hyperleptinemia that is likely the result of desensitization of the leptin signal as noted elsewhere [35]. This had reflected the development of leptin resistance occurring on at least two distinct levels involving saturated transport of leptin across the blood-brain barrier and the abnormalities in extent of leptin receptor activation and/or signal transduction [36]. The beneficial role of nigella sativa that enhances glucose-induced insulin release and improves glucose tolerance was evident among the mechanisms of the hypoglycemic and immune potentiating effects [37].

Thymoquinone (active constituent of nigella sativa seeds) which was reported to prevent oxidative stress injury in hepatocytes [38]. This was confirmed here by the reduction of MDA and oxLDL that subsequently prevent lipid peroxidation, fibrogenesis and atherogenesis. Owing to nigella sativa free radical scavenging properties verified by subsequent upraising of AOC%, it exhibited a protection from the fibrogenic stimuli(confirmed by reducing fibronectin increments), and anti-inflammatory effects (associated decrease of NF-κB/p65 mRNA expression levels), which aligned withthe reported inhibition of eicosanoid synthesis [39]. In addition to that, nigella sativa complements its immune-modulatory potential by enhancing cell mediated and humoral immunity [40] that was reflected by the monitored anti- inflammatory and anti-oxidant potential of NS outcome of combined therapy in minimizing the susceptibility to membrane lipid peroxidation and fibrogenic liability [41], which was verified by the reduction of MDA, oxLDL and fibronectin.

Furthermore, the reduction of BMI here in (group IV> group III) aligned with the outcome of chromium picolinate supplements which was noted to influence glucose tolerance and lipid profile and enhance insulin activity [42]. Concordantly, as evident by reduction of BMI and WHR, the rationale for decreasing appetite via promoting weight loss to be fat rather than lean tissue via the effect of chromium supplements was reported previously [43]. Moreover, the additional impact of green tea consumption bygroup III and group IV reflected an increased energy expenditure influencing thermogenesis and fat metabolism besides affecting lipase activity which may promote weight loss as noted elsewhere [44]. Also,

the antioxidant power of green tea adds beneficial use in ameliorating the obesity status monitored here [45]. Moreover, the anti-inflammatory potential exhibited by supplementation of omega-3 fatty acids plus approved in group IV > group III was in agreement with report by López-Vicario et al. [46]. In addition, it agreed with previous reports indicating its influence on postprandial satiety which promotes weight reduction [47], confirmed by lowering of BMI and WHR in (groupIV> groupIII). Also, it aided in reducing insulin resistance and decreasing the liver fat content and lipid oxidation as previously reported by González-Périz et al. [48]. Long chain omega-3 polyunsaturated fatty acid promote an increase in gene expression of peroxisomal fatty acid oxidation in liver and muscle [49], besides an increase in the expression of uncoupling proteins contributing to increased loss of energy in the form of heat and less storage of energy in the form of fat [50] leading to reduction of the BMI observed here. It is associated with increased adiponectin [51] and decreased glucose and HOMA-IR as observed here in alongside the reported decreased triglycerides as noted elsewhere [52].

5. Conclusion

Assessment of biomarkers for monitoring the correction of NAFLD included adipokines, cytokines, anti-oxidants, indices of glycemic status, fibrogenesis and lipid oxidation, defined the role of management strategy at the cross-road of energy homeostasis with fibrogenic and atherogenic stimuli by replenishing anti-oxidant, anti-inflammatory and immune-modulatory mechanisms.

Thereby, for coping with multivariate metabolic derangement in NAFLD, caloric restricted diet, exercise and complex strategies of the provided natural product supplements (nigella sativa, omega-3 fatty acids, green tea) together with chromium picolinate consumption for six months was established, targeting the complexity of obesity related oxidative stress induced signaling pathways via integrated signals by metabolic sensing neurons affecting glycemic control, hormonal outputs, cytokines expression and balancing adiponectin/ leptin/ insulin axis, which potentiate a net of catabolic state leading to weight loss.

References

[1] Sakaguchi S, Takahashi S, Sasaki T, Kumagai T, Nagata K. Progression of alcoholic and non-alcoholic steatohepatitis: common metabolic aspects of innate immune system and oxidative stress. Drug Metab Pharmacokinet. 2011; 26(1):30-46.

[2] Gaudio E, Nobili V, Franchitto A, Onori P, Carpino G. Non-alcoholic fatty liver disease and atherosclerosis. Intern Emerg Med. 2012; 3:S297-305.

[3] Sookoian S, Gianotti TF, Rosselli MS, Burgueño AL, Castaño GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human non-alcoholic fatty liver disease. Atherosclerosis. 2011; 218(2):378-85.

[4] Sáinz N, González-Navarro CJ, Martínez JA, Moreno-Aliaga MJ. Leptin signaling as a therapeutic target of obesity.Expert Opin Ther Targets. 2015;1:1-17.

[5] Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. Annu Rev Physiol. 2010; 72:219-46.

[6] Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012; 148(5):852-71.

[7] Paolisso G, Ammendola S, Del Buono A, Gambardella A, Riondino M, Tagliamonte MR,et al. Serum levels of insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 in healthy centenarians: relationship with plasma leptin and lipid concentrations, insulin action, and cognitive function. J Clin Endocrinol Metab. 1997; 82(7):2204-9.

[8] Lashinger LM, Ford NA, Hursting SD. Interacting inflammatory and growth factor signals underlie the obesity-cancer link. J Nutr. 2014; 144(2):109-13.

[9] Tilg H, Moschen AR. Evolution of inflammation in non-alcoholic fatty liver disease: the multiple parallel hits hypothesis.Hepatology. 2010; 52(5):1836-46.

[10] Marra F, Bertolani C. Adipokines in liver diseases. Hepatology. 2009; 50(3):957-69.

[11] Van Loo G, Beyaert R. Negative regulation of NF-κB and its involvement in rheumatoid arthritis.Arthritis Res Ther. 2011;13(3):221.

[12] Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. J Chronic Dis. 1972; 25(6):329-43.

[13] Garrow JS, Webster J. Quetelet's index (W/H2) as a measure of fatness. Int J Obes. 1985; 9(2):147-53.

[14] Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol. 1969; 22(2):246.

[15] Fenzl A, Itariu BK, Kosi L, Fritzer-Szekeres M, Kautzky-Willer A, Stulnig TM, Kiefer FW. Circulating betatrophin correlates with atherogenic lipid profiles but not with glucose and insulin levels in insulin-resistant individuals.Diabetologia. 2014;57(6):1204-8.

[16] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.Diabetologia. 1985; 28(7):412-9.

[17] Al Maskari MY, Alnaqdy AA. Correlation between Serum Leptin Levels, Body Mass Index and Obesity in Omanis. Sultan Qaboos Univ Med J.2006 ;6(2):27-31.

[18] Robaczyk MG. Evaluation of leptin levels in plasma and their reliance on other hormonal factors affecting tissue fat levels in people with various levels of endogenous cotisol. Ann Acad Med Stetin. 2002; 48: 283-300.

[19] Won WY, Lee CU, Chae JH, Kim JJ, Lee C, Kim DJ. Changes of plasma adiponectin levels after smoking cessation. Psychiatry Investig. 2014; 11(2):173-8.

[20] Stubbs TM, Lazarchick J, horger EO. Plasmafibronectin levels in preclampsia. Am J obstetric Gynecol. 1984; 150:885-7.

[21] Samani KG, Farrokhi E. Effects of cumin extract on oxLDL, paraoxanase-1 activity, FBS, total cholesterol, triglycerides, HDL-C, LDL-C, Apo A1, and Apo B in in the patients with hypercholesterolemia. Int J Health Sci (Qassim). 2014; 8(1):39-43.

[22] Manuel PP, Miguel A. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Analyt Biochem 1999; 259: 337-341.

[23] Ohkawa, H., Ohishi, N., Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem.1979; 95: 351-358.

[24] Slade HB, Greenwood JH, Hudson JL, Beekman RH 3rd, Riedy MC, Schwartz SA. Lymphocyte phenotyping of infants with congenital heart disease: comparison of cell preparation techniques. Digan Clin Immunol.1988; 5(5): 249-55

[25] Sun W, Guo MM, Han P, Lin JZ, Liang FY, Tan GM, et al. Id-1 and p65 subunit of NF-kB promote migration of nasopharyngeal carcinoma cells and are correlated with poor prognosis. Carcinogenesis. 2012; 33(4):810-7.

[26] Eslamparast T, Eghtesad S, Poustchi H, Hekmatdoost A. Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease.World J Hepatol. 2015; 7(2):204-12.

[27] Gambino R, Musso G, Cassader M. Redox balance in the pathogenesis of non-alcoholic fatty liver disease: mechanisms and therapeutic opportunities. Antioxid Redox Signal. 2011; 15(5): 1325-65.

[28] Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: diversity, patterns of recognition, and pathophysiology.Antioxid Redox Signal. 2010; 13(1): 39-75.

[29] Kynde I, Heitmann BL, Bygbjerg IC, Andersen LB, Helge JW. Hypoadiponectinemia in overweight children contributes to a negative metabolic risk profile 6 years later. Metabolism. 2009; 58(12): 1817-24.

[30] Ley K, Miller YI, Hedrick CC. Monocyte and macrophage dynamics during atherogenesis. Arterioscler Thromb Vasc Biol. 2011; 31: 1506-1516.

[31] Skoien R, Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, et al. Heterogeneity of fibrosis patterns in non-alcoholic fatty liver disease supports the presence of multiple fibrogenic pathways. Liver Int. 2013; 33(4):624-32.

[32] van der Meer AJ, Sonneveld M, Schouten JN, Janssen HL. [Reversibility of hepatic fibrosis]. Ned Tijdschr Geneeskd. 2014;158: A6790.

[33] Hursting SD, Dunlap SM, Ford NA, Hursting MJ, Lashinger LM. Calorie restriction and cancer prevention: a mechanistic perspective.Cancer Metab. 2013; 1(1):10.

[34] Hivert MF, Langlois MF, Carpentier AC.The entero-insular axis and adipose tissue-related factors in the prediction of weight gain in humans. Int J Obes (Lond). 2007 ; 31(5): 731-42.

[35] Moyse E, Bédard K, Segura S, Mahaut S, Tardivel C, Ferland G,et al. Effects of aging and caloric restriction on brainstem satiety center signals in rats. Mech Ageing Dev. 2012; 133(2-3):83-91.

[36] Han Y, Joe Y, Seo E, Lee SR, Park MK, Lee HJ, Kim DK.The hyperleptinemia and ObRb expression in hyperphagic obese rats. Biochem Biophys Res Commun. 2010; 394(1):70-4.

[37] Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013; 3(5):337-52.

[38] Ibrahim RM, Hamdan NS, Ismail M, Saini SM, Abd Rashid SN, Abd Latiff L, Mahmud R. Protective Effects of Nigella sativa on Metabolic Syndrome in Menopausal Women. Adv Pharm Bull. 2014; 4(1):29-33.

[39] Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor-kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. 2008; 6(6):1059-70.

[40] Al-Suhaimi EA. Hepatoprotective and immunological functions of Nigella sativa seed oil against hypervitaminosis A in adult male rats. Int J Vitam Nutr Res. 2012; 82(4):288-97.

[41] Sharma NK, Ahirwar D, Jhade D, Gupta S. Medicinal and Phamacological Potential of Nigella sativa: A Review. Ethnobotanical Review 13: 946-55, 2009.

[42] Suksomboon N, Poolsup N, Yuwanakorn A. Systematic review and meta-analysis of the efficacy and safety of chromium supplementation in diabetes. J Clin Pharm Ther. 2014; 39 (3): 292-306.

[43] Chen WY, Chen CJ, Liu CH, Mao FC. Chromium attenuates high-fat diet-induced non-alcoholic fatty liver disease in KK/HlJ mice. Biochem Biophys Res Commun. 2010; 397(3): 459-64.

[44] Heber D, Zhang Y, Yang J, Ma JE, Henning SM, Li Z. Green tea, black tea, and oolong tea polyphenols reduce visceral fat and inflammation in mice fed high-fat, high-sucrose obesogenic diets. J Nutr. 2014; 144(9):1385-93.

[45] Xu Y, Zhang M, Wu T, Dai SXu J, Zhou Z. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct. 2015; 6(1): 297-304.

[46] López-Vicario C, Alcaraz-Quiles J, García-Alonso V, Rius B, Hwang SH, Titos E, et al. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. Proc Natl Acad Sci U S A. 2015; 112(2): 536-41.

[47] Simopoulos AP. Dietary omega-3 fatty acid deficiency and high fructose intake in the development of metabolic syndrome, brain metabolic abnormalities, and non-alcoholic fatty liver disease. Nutrients. 2013; 5(8):2901-23.

[48] González-Périz A, Horrillo R, Ferré N, Gronert K, Dong B, Morán-Salvador E, et al. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J. 2009; 23(6): 1946-57.

[49] Tai CC, Ding ST. N-3 polyunsaturated fatty acids regulate lipid metabolism through several inflammation mediators: mechanisms and implications for obesity prevention. J Nutr Biochem. 2010; 21(5): 357-63.

[50] Philp LK, Heilbronn LK, Janovska A, Wittert GA. Dietary enrichment with fish oil prevents high fat-induced metabolic dysfunction in skeletal muscle in mice. PLoS One. 2015; 10(2): e0117494.

[51] Ding WJ, Wang Y, Fan JG. Regulation of adipokines by polyunsaturated fatty acids in a rat model of non-alcoholic steatohepatitis. Arch Iran Med. 2014; 17(8): 563-8.

[52] Rafraf M, Mohammadi E, Asghari-Jafarabadi M, Farzadi L.Omega-3 fatty acids improve glucose metabolism without effects on obesity values and serum visfatin levels in women with polycystic ovary syndrome. J Am Coll Nutr. 2012; 31(5): 361-8.