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Ameliorative Effect of probiotics on Anti-inflammatory markers [TNFα, IL6] on hepatic Lipid Metabolism Experimentally Induced Non Alcoholic Fatty Liver Disease in Rats

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ABSTRACT

The main study applied on the biochemical effects of probiotics on hepatic Lipid Metabolism experimentally induced Nonalcoholic fatty liver disease [NAFLD] in rats. Thirty male albino rats were divided into three groups [10 rats each]. The first group fed a normal diet and represents the control group. The second group [NAFLD] fed normal diet enriched with1% cholesterol and 2% coconut oil and act as positive control [+ ve control]. The third group fed on normal diet enriched with1% cholesterol and 2% coconut oil and probiotics [BIO-BC™] at a dose of 1-2g/liter at a rat dose[0.5-1ml/kg. body. weight]. Samples collected after 2,4and6 weeks after induction from treatment. Serum was collected for estimation of serum Tumor necrosis factor alpha [TNFα] , Interlukein-6 [IL-6] .Our results showed a significant increase in serum TNFα, IL-6. The behavioral biochemical results indicated treatment with probiotics showed significant changes and improved these parameters.

Keywords: NAFLD, Probiotics, Pro-inflammatory cytokines.

1. INTRODUCTION

NAFLD was considered a benign condition, but is now increasingly recognized as a major cause of liver-related morbidity and mortality. The fundamental derangement in nonalcoholic fatty liver disease is insulin resistance, a key component of the metabolic syndrome, which includes type 2 diabetes mellitus, hypertriglyceridemia, essential hypertension, low circulating high-density lipoprotein, and obesity [1].Pro-inflammatory cytokines, including TNF-αare thought to play an important role in vascular inflammation, leading to atherosclerosis and development of coronary arterial disease [2].

Among several mediators cytokines and chemokines might play a pivotal active role in NAFLD and are considered as potential therapeutic targets. The evidence on the potential role of cytokines and chemokines in the Non Alcoholic Fatty Liver Disease [NAFLD].Pro-inflammatory cytokines shift plays an important role in vascular regulatory mechanisms [3]. "probiotics" the concept had been around from the beginning of the 20th century. As years have gone by the meaning of this term has changed [4]. The aim of this study to Evaluate some anti-inflammatory effect of probiotics on Hepatic Lipid Metabolism in Experimentally Induced NAFLD rats.

2. MATERIALS AND METHODS

2.1 Animals and chemicals:

Male white albino rats, 6weeks age and weighting [150– 180g] were used in the experiment. Rats were housed in separate metal cage with free access to water. Rats were kept under constant and nutritional environmental condition throughout the experiment. Rats were left for15 days before beginning of experiment for acclimatization. Cholesterol and coconut oil were purchased from El-Goumhouria Co. for Trading Chemicals, Egypt. Hepatic lipid metabolism

induced NAFLD by continuous supplementation of high fat diet [HFD] was prepared by High Cholesterol [1% wt/wt] and [Coconut oil 2%wt/wt] to normal ratio according to [5].

2.2 Probiotic [BIO-BC™]:
Improve growth this product was kindly supplied from Animal Health Division by Kanzy Medipharm[™],Egypt Daone chemical co. Ltd ./Da405, Daon Sihwa industrial complex, 1252.6,Jeongwang-dong,Siheung-si, Kyonggi-do, Korea . It was given orally in dose and duration as mentioned below:

2.2.A Composition [Each kg contains]: It is composed of:
*Streptococcus faecalis*1billionCFU/kg.
Bacillus subtilis 0.2billionCFU/kg.
Clostridium butyricum 0.2billionCFU/kg.
Carrier glucose 1kg

2.2.B Preparation and dosage of probiotic:
It is a powder added to drinking water in a dose of 1-2g/liter [Kanzy Medipharm] as manufacturer instructions and at a rat dose [0.5-1ml/kg. body. weight] orally according to [6].

2.3 Experimental design:
Rats were divided into 3 groups [10 per each].
Group I was fed on normal diet and served as control group.
Group II was fed on high fat diet [Normal NAFLD] for 12 weeks.
Group III was fed on normal diet [NAFLD] and probiotics at a dose of1-2g/liter [Kanzy Medipharm] as manufacturer instructions and at a rat dose [0.5-1ml/kg. body. weight] daily according to [6].

2.4 Sampling:
After overnight fasting blood samples was collected from all animal groups[control and experimental groups] after12weeks for detection of hyperlipidemia .then samples were collected after 2,4and6 weeks from onset of treatment.

*2.4.1 Blood samples***:**
Blood samples were collected from medial canthus of eye and collected in dry, clean and screw capped tubes then rats decapitated for liver tissue removal containing serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clean clear serum was separated by Pasteur pipette and kept in a deep freeze at -20C till used for determination of the biochemical Parameters: Serum Pro-inflammatory cytokines as serum TNFα[7], IL-6[8].

2.5 Statistical analysis:
The obtained data were analyzed using the statistical package for social science [SPSS, 13.0 software, 2009] [9] for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping.

3. RESULTS AND DISCUSSION

The obtained results demonstrated in tables [1, 2 and 3] found that A significant increase in TNF-α **,** IL-6 concentration was observed in hepatic lipidmetabolism Experimentally induced NAFLD in rats after 2,4 and 6weeks compared with the normal control group. These results were nearly similar to [10] suggests that A significant increase in serumTNF-α **,**IL-6concentration may be due to the inflammatory cytokine TNF [tumor necrosis factor]-*α* plays a pivotal role in the disruption of macrovascular and microvascular circulation both in vivo and in vitro. AGEs [advanced glycation end-products]/RAGE [receptor for AGEs], LOX-1 [lectin-like oxidized low-density lipoprotein receptor-1] and NF-*κ*B[nuclear factor *κ*B] signaling play key roles in TNF-*α* expression through an increase in circulating and/or local vascular TNF-*α* production. The increase in TNF-*α* expression induces the production of ROS [reactive oxygen species], resulting in endothelial dysfunction in many pathophysiological conditions. Lipid metabolism, dietary supplements and physical activity affect TNF-*α* expression. The interaction between TNF-*α* and stem cells is also important in terms of vascular repair or regeneration [11].

Table 1. Effect of Probiotics administration on some pro-inflammatory cytokines after 2weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	**TNF-α pg/ml**	**IL-6 pg/mL**
Normal Control group	10.71^{e} ±1.06	43.87^{ab} ±1.51
control NAFLD group	51.27^{a} ±2.23	149.36^{ab} ±4.19
NAFLD Treated probiotics group	20.99^{cd} ±3.34	130.26^{b} ±5.51

Data are presented as (Mean ± S.E).S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

Table 2. Effect of Probiotics administration on some pro-inflammatory cytokines after 4weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	TNF-α(pg/ml	IL-6 pg/mL
Normal Control group	20.10^{cd} ±2.06	41.15^{e} ±2.85
control NAFLD group	39.14^{b} ±1.42	165.91^{a} ±7.62
NAFLD Treated probiotics group	22.94^{cd} ±1.12	85.09^{c} ±1.81

Data are presented as (Mean ± S.E).S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

Table 3. Effect of Probiotics administration on some pro-inflammatory cytokines after 6weeks on Hepatic Lipid Metabolism Experimentally Induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	**TNF-α pg/ml**	**IL-6 pg/mL**
Normal Control group	12.86^{e} ±2.14	63.86^{a} ±3.19
control NAFLD group	49.31^{a} ±4.65	127.87^{bc} ±11.96
NAFLD Treated probiotics group	23.90^{cd} ±2.73	26.87^{efghi} ±4.25

Data are presented as (Mean ± S.E).S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at *(P<0.05*)

In addition [12] observed that a significant increase in serum TNF-*α* may be due to the high-fat meal, both triacylglycerol and TNF-*α* levels increased more in subjects with the metabolic syndrome [13].

Also [14] illustrated that a significant increase in serum TNF-*α* A high-cholesterol diet induces high levels of serum TNF-*α* concentration, whereas the mRNA expression. The leptin axis has functional interactions with elements of metabolism, such as insulin, and inflammation, including mediators of innate immunity, such as interleukin-6. Leptin resistance and its interactions with metabolic and inflammatory factors, therefore, represent potential novel diagnostic and therapeutic targets in obesity-related cardiovascular disease [15].

Moreover [16] stated that a significant increase in serum IL-6 is a polyvalent cytokine with pro-inflammatory and pro-oncogenic activity, and it supports hematopoiesis and is a predictive marker of insulin resistance and cardiovascular diseases hepatic and serum IL-6 levels are higher in NAFLD.

Treatment with Probiotics found that a significant decrease in serum TNF-α **,** IL-6 concentration on hepatic lipid metabolism experimentally induced NAFLD in rats after 2,4and 6 weeks compared with control NAFLD groupThese results were nearly similar to[17] found that a significant decrease in serum IL-6, as well as leptin, activates AMP-activated protein kinase[AMPK] in skeletal muscle and adipose tissue. Leptin is a lipolytic hormone and pro-inflammatory cytokine with important effects in regulating body weight, metabolism, and reproductive function. Consistent with AMPK activation, IL-6 increases fat oxidation in vitro, ex vivo.

Therefore [18] observed that a significant decrease in serum TNF-*α* may be due to dietary supplements and exercise favorably reduce the risk of vascular dysfunction by inhibiting TNF-*α* production and [or] TNF-*α*-mediated signaling also The role of TNF-α in NAFLD may be due to its capacity to induce hepatocytes apoptosis, insulin resistance and to regulate KC activation locally Moreover, TNF-α regulates hepatic lipid metabolism [19].

The present study demonstrated that probiotics treatment provided an effective treatment against NAFLD in rats since these compounds were able to ameliorate serum biochemical parameters.

4. CONCLUSION

Administration of diet rich in the natural products as probiotics is very important for treatment of different body organs, especially liver against NAFLD and Inflammation.

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