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Beneficial effect of probiotics on antioxidants and Oxidative Stress in experimental induced Non Alcoholic Fatty Liver Disease in Rats

Omayma A.R. Abou Zaid, Omnia M. AbdEl-hamid and Shimaa Ahmed El-Refaey Atwa

Department of Biochemistry, Faculty of Veterinary Medicine, Moshtohor, Benha University.

* Corresponding author: Omayma A.R. Abou Zaid; email: omayma_ragab55@yahoo.com

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ABSTRACT

The main objective of this study to investigate the Beneficial effect of probiotics on Oxidative Stress in experimental 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 group [+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] orally . Samples collected after 2,4and6 weeks after induction from treatment. serum was collected for estimation of Serum Nitric Oxide [NO], Serum L- Malonialdehyde [L-MDA] and Tissues as liver [Enzymatic Antioxidants as [Liver Catalase [L-CAT, Liver superoxide dismutase [L-SOD] ,Non Enzymatic Antioxidants as Liver Reduced Glutathione [L-GSH]. Our results revealed a significant decrease in serum NO, L-CAT, L-SOD, L-GSH moreover serum L-MDA. The behavioural biochemical results indicated treatment with probiotics showed significant changes and improves these parameters.

Keywords: NAFLD, probiotics, Antioxidants, Histopathological.

1. INTRODUCTION

Nonalcoholic fatty liver disease [NAFLD] is now recognized as the most common cause of cryptogenic cirrhosis [1]. Oxidative stress from steatotic hepatocytes leads to lipid peroxidation, impaired mitochondrial and peroxisomal oxidation of fatty acids, and cytokine release [2].

The term “probiotics” was created around, but the concept had been around from the beginning of the 20th century. As years have gone by the meaning of this term has changed. However the definition given by [the Joint Food and Agriculture Organization/World Health Organization Working Group in 2001] [3].

The aim of this study to alter ate the beneficial effect of probiotics on oxidative stress in experimental induced NAFLD in rats.

2. MATERIALS AND METHODS

2.1 Animals and chemicals:

Male white albino rats, 6-8weeks 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 ration according to [4].

2.2 Probiotic(BIO-BC™):

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 contain]: 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 manufactures instructions and at a rat dose [0.5-1ml/kg. body. weight] orally .according to [5].

2.3 Experimental design:

Rats were divided into 3 groups [10 per each] main groups placed in individual cages and classified as follow:

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 high fat diet [NAFLD] and treated probiotics at a dose of1-2g/liter [Kanzy Medipharm] as manufactures instructions and at a rat dose [0.5-1ml/kg. body. weight] according to [5] orally.

2.4 Sampling:

After overnight fasting blood samples was collected from all animal groups [control and experimental groups] after12 weeks for detection of NAFLD [hyperlipidemia] .then samples were collected after 2,4and6 weeks from onset of treatment.

2.4.1 Blood samples:

Blood Samples were collected from Medial Canthes 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 NO [6], Serum L-MDA [7] and tissues as L-CAT[8], L-SOD[9], L-GSH [10].

2.4.2 Tissue Sample:

Liver specimens were preserved in 10% buffered neutral formalin and subjected for Histopathological Examination according to the technique described by [11].

2.5 Statistical analysis:

The obtained data were analyzed represented using the statistical package for social science [SPSS, 13.0 software, 2009] [12] 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.

Table1. Effect of probiotics on serum nitric oxide [NO] , L-MDA, tissue L-CAT L-SOD L-GSH After 2 weeks In Experimental induced Nonalcoholic fatty liver disease in rats

Parameter/ Group	NO m mol/l	L- MDAn mol/l	L-CAT gm/tissue	L-GSH ng /gm/tissue	L-SOD U/gm
Normal Control group	112.13^{a} ±4.11	48.83^{e} ±4.60	56.34^{eb} ±3.93	10.03^{a} ±0.37	45.81^{a} ±0.96
control NAFLD group	72.86^{e} ±12.46	173.36^{a} ±3.23	40.96^{cde} ±0.98	5.68^{ef} ±0.39	22.35^{efg} ±5.38
NAFLDTreated probiotics group	86.21^{de} ±2.61	86.35^{bc} ±1.59	44.11^{c} ±2.80	7.31^{cd} ±0.13	25.48^{efg} ±2.88

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 Treatment on serum nitric oxide [NO], L-MDA, tissue L-CAT L-SOD L-GSH After4 weeks in experimental induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	NO m mol/l	L-MDA nmol/l	L-CAT gm/tissue	L-GSH ng /gm/tissue	L-SOD U/gm
Normal Control group	107.01^{abc} ±5.13	51.85^{e} ±6.32	64.25^{a} ±1.68	8.24^{b} ±0.38	32.96^{cde} ±1.98
control NAFLD group	83.59^{de} ±14.90	139.98^{bc} ±14.36	34.99^{ef} ±3.44	6.58^{de} ±0.20	30.09^{de} ±5.14
NAFLD Treated probiotics group	89.28^{bcde} ±1.31	99.56^{b} ±7.01	52.73^{b} ±1.62	7.33^{cd} ±0.48	10.76^{i} ±0.96

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 Treatment on serum nitric oxide [NO] , L-MDA, tissue L-CAT, L-SOD, L-GSH after6 weeks in experimental induced Nonalcoholic fatty liver disease in rats.

Parameter/ group	NO m mol/l	L- MDA nmol/l	L-CAT gm/tissue	L-GSH ng /gm/tissue	L-SOD U/gm
Normal Control group	107.79^{abc} ±3.17	61.77^{de} ±3.48	55.49^{ab} ±3.10	6.78^{b} ±0.26	42.31^{ab} ±1.95
control NAFLD group	87.39^{cde} ±8.79	127.35^{a} ±5.50	39.07^{f} ±3.03	6.88^{ef} ±0.17	24.37^{efg} ±6.10
NAFLD Treated probiotics group	88.29^{bcde} ±2.77	26.82^{e} ±3.09	35.28^{def} ±1.96	7.32^{g} ±0.10	31.45^{de} ±0.58

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]*

3. RESULTS AND DISCUSSION

The obtained results demonstrated in tables [1, 2 and 3] revealed that a significant decrease in serum nitric oxide in rats after 2,4 and 6weeks compared with the normal control group These results were nearly similar to [13]; [14] stated that a significant decrease in serum nitric oxide may be due to the various functions of ECs (endothelial cells) such as the control of fibrinolysis, coagulation, vascular tone ,growth and immune response. In addition to the citrulline, NO cycle is regulated by ASS [arginine succinate synthase). NO is synthesized from the conversion of l-arginine into l-citrulline mediated by eNOS, and ASS catalyses the rate-limiting step in the arginine regeneration through the citrulline/NO cycle and appears to be regulated with eNOS activity [15] .

Also [16] stated that a significant decrease in serum NO may be due to the Endothelial dysfunction associated with TNF-α in pathophysiological conditions is linked to excess production of ROS and a decrease in NO bioavailability and TNF-α appears to decrease the bioavailability of NO by (i) diminishing the production of NO the main pro-inflammatory cytokine. More ever increased the basal bioavailability of the vasoconstrictor prostaglandin and reduced the basal bioavailability of NO.

Treatment with Probiotics exhibited a significant increase in serum NO concentration in rats after 2,4and 6 weeks compared with control NAFLD group. These results were in accordance with [17] revealed that a significant increase in serum NO may be due to hydrogen peroxide, nitric oxide, and short chain fatty acids [SCFA], such as lactic and acetic acids, which reduce the pH of the lumen . SCFA can disrupt the outer membranes of gram-negative pathogens causing inhibition of pathogen growth. Bacteriocins can either permeabilize the inner membrane of gram-negative bacteria, leading to disruption and formation of pores. Microcins [produced by gram negative bacteria], on the other hand, can target the inner membrane, enzymes that are involved in DNA or RNA structure and synthesis, or protein synthesis enzymes [18].

The obtained results demonstrated in tables [1, 2 and 3] reported that a significant increase in serum L-MDA after 2,4 and 6weeks compared with the normal control group. these results were nearly similar to [19] found that a significant increase in serum L-MDA may be due to Lipid peroxidation One case of oxidative stress.

Moreover [20] found that a significant increase in serum L-MDA may be due to due to Liver fat accumulation and insulin resistance characterize the first hit and a responsible for the development of steatosis. The main factors initiating the second hit are oxidative stress and subsequent lipidperoxidation, together with the production of pro-inflammatory cytokines, principally tumor necrosis factor [TNF]a and hormones derived from adipose tissue.

Treatment with Probiotics exhibited a significant decrease in serum L- MDA concentration in rats after 2,4and 6 weeks compared with control NAFLD group. These results were nearly similar to [20] revealed that significant decrease in serum L- MDA may be due to the probiotics affects plasma levels of cytokines and oxidative/nitrosative stress parameters, it significantly improved plasma levels of MDA. the Probiotics are live microorganisms which have favorable physiological, biochemical and immune effects by improving the functions of the normal intestinal microflora.

The obtained results demonstrated in tables[1,2and3] recorded that a significant decrease in L-CAT, L-GSH, L-SOD concentration in rats after 2, 4 and 6weeks compared with the normal control group. these results were nearly similar to [21] showed that a significant decrease in L-CAT, L- GSH , L-SOD may be due to Antioxidants in our body are the first to fight free radicals .The initial encounter leads to neutralization of free radical. Yet, another free radical is formed in this process. This begins a chain reaction. Before other free radicals are neutralized, thousands of reactions take place instantly.

Moreover [22] a significant decrease in L-CAT, L-SOD may be due to Oxidative stress –is seen when the creation of ROS in a system surpasses its ability to neutralize and eradicate them. This may be the result of an absence of anti-oxidation ability due to its disruption in production or distribution. It may also be

a result of excess ROS caused by environmental factors or high risk .The following are among the factors that may contribute to an increase in the body's oxidant.

In addition to [23] stated that hyperlipidaemia and hyperglycaemia are among a myriad of risk factors that may contribute to the pathogenesis of many cardiovascular diseases, such as hypertension, diabetes and atherosclerosis.

Treatment with Probiotics exhibited a significant increase after 2, 4 weeks, a significant decrease after 6 weeks in L-CAT, ,exhibited a significant increase in L-GSH after 2, 4 and 6 weeks , a non-significant increase after 2weeks , a significant decrease after 4 weeks , a significant increase after 6 weeks in L-SOD concentration in rats after 2, 4 and 6 weeks compared with control NAFLD group. these results were nearly similar to [24] revealed that a significant decrease may be due to Lipotoxicity, oxidative stress, cytokines, and other pro-inflammatory mediators may each play a role in transition of steatosis to NASH. The present "gold standard" management of NASH is modest weight reduction, particularly correction of central obesity achieved by combining dietary measures with increased physical activity.

Also [25] found that a significant increase in L-CAT, L-SOD may be due to reactive oxygen species [ROS]. It is critical to understand not only the triggers for hepatitis [injury and inflammation] in NASH but also how this is perpetuated as chronic liver disease. The present focus is on whether the biochemical processes that generate oxidative stress lead to hepatocyte injury and secondary recruitment of inflammation or whether inflammation is the primary mediator of liver cell injury. Insulin resistance is a reproducible pathogenic factor in NASH.

Moreover [26] revealed that hydrogen peroxide, nitric oxide, and short chain fatty acids (SCFA), such as lactic and acetic acids, which reduce the pH of the lumen . SCFA can disrupt the outer membranes of gram-negative pathogens causing inhibition of pathogen growth . Bacteriocins can either permeabilize the inner membrane of gram-negative bacteria, leading to disruption and formation of pores. Microcins [produced by gram negative bacteria], on the other hand, can target the inner membrane, enzymes that are involved in DNA or RNA structure and synthesis, or protein synthesis enzymes.

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, antioxidants, Immunoglobulins and prevent the Lipid Peroxidation in the serum and regulate Liver Function Tests.

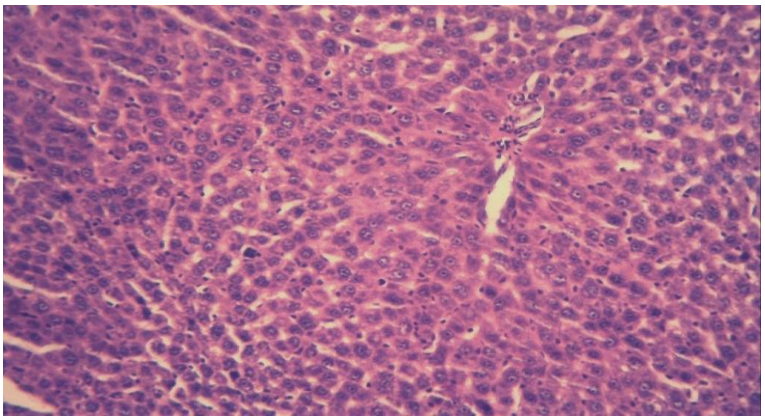

Figure 1: Liver of Normal control rats showing Normal Histological structure of the liver, Normal Hepatic Lobules and Hepatocytes.

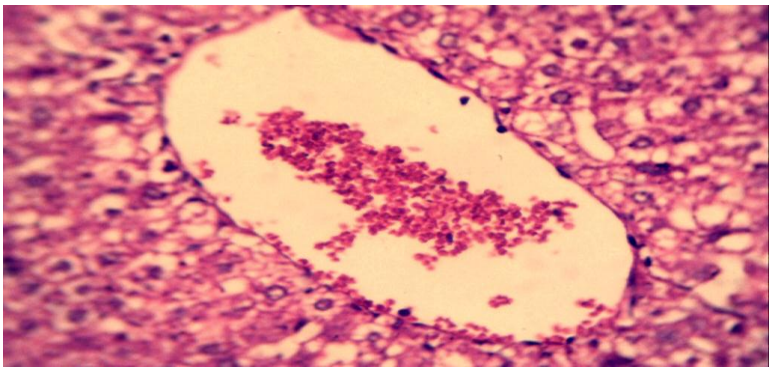

Figure 2: Liver of NAFLD rats, showing severe congestion of the central vein and portal blood vessels. The portal area showed mild hyperplasia of the epithelial cell lining of bile duct and mild fibrous tissue proliferation with Sever dilatation of central vein.

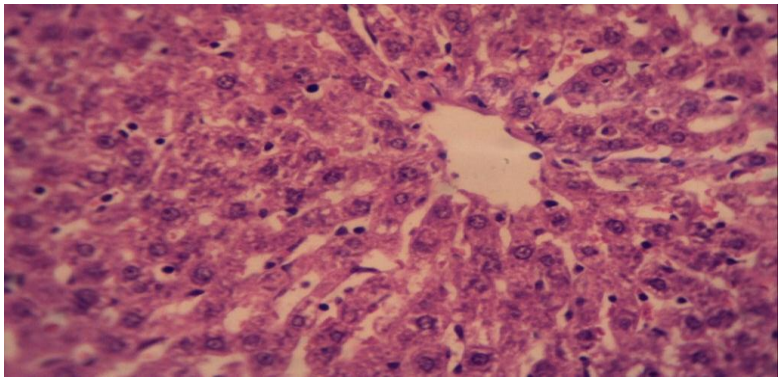

Figure 3: Liver of Probiotics rats followed by NAFLD , showing mild hydro pic degeneration of hepatocytes and mild congestion of hepatocytes central vein portal blood vessels mononuclear leucocytic infiltration in the portal area. Some of hepatocytes showing scatter hepatocytes severed from fatty change.

4. CONCLUSION

So we conclude that, administration of diet rich in the natural products as probiotics is very important for treatment of different body organs, especially liver against oxidative stress.

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