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# EFFECT OF SOME PROBIOTIC STRAINS ON LIPID METABOLISM

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## SUMMARY

In the present study, Fifteen selected probiotic strains of lactic acid bacteria as (lactobacillus, Streptococcus and Bifidobacterium spp) were examined for their ability to remove cholesterol (T-Ch) and triglycerides (TG) from laboratory media during their growth. All selected strains could remove T-Ch and triglycerides (TG) on different media as MRS-THIO broth media GM-THIO growth media except 2 strains only. A combination between the probiotic strains used was carried out to select the proper mixture in removing lipids. Derived yoghurt from the best mixture was analysed for T-Ch and TG under the manufacture conditions proved the ability of chosen mixture in removing lipids.

The study extended to evaluate, the effect of yoghurt manufactured by probiotic strains (1% starter ) on the lipid profile through experimental hyperlipidemic rats (rats fed on 1% cholesterol and 0.5% of cholic acid dissolved in 20% coconut oil ration for 45 days). The hyperlipidemic rats were subdivided into four groups; G1, fed on rotion free probiotics served as (-) ve control group, G2, 3, 4 fed on ration containing different groups of strains from probiotics, *L.acidophillus* N10, Str.cremoris F 99 and Bif.ther-bifidum99 (B group); *L.acidophillus* N10, *L.bulgaricus* N5 and *Str.thermophillus* S1 (H group) ; *L.acidophillus* N10, Str.cremoris F50, L.bulgaricus N5 and Bif-longum K69 (E group). The data showed that all the tested groups have similar significant reduction in serum T-Ch, LDL-Ch and TG as well as significant increase in the level of HDL-Ch . All the tested yoghurt showed significant reduction in serum total fatty acids (TFA). The most reduction effecton fatty acids were obtained by the group H which could restore the ratio of unsaturated fatty

acids / saturated ones to become near to the control value. It is worth mentioning that the biological studies confirmed with the aorta tissues histobiology.

**Keyword:** cholesterol, Triglycerides, rats, Yoghurt, TFA, SFA, UFA, probiotic bacteria, aorta.

## INTRODUCTION

A high level of serum total cholesterol in generally considered to be a risk factor for coronary heart disease and atherosclerosis (Moore, 1996 and Yamamoto et al., 1999). Reducing the level cholesterol causing decreases in incidence and mortality of ischemic heart disease and atherosclerosis (Mabuchi, 1999 and Suzan 2003). As such, much attention has been drowning to different dietary ways of reducing the serum total cholesterol level. Many studies has been performed in experimental animals to elucidate the effect of fermented dairy products on serum cholesterol especially with selected strain of lactic acids bacteria (St-Onge et al., 2000). Yoghurt is indicated as the best diet for balancing and preserving the biological functions of the gut. All these biological effects of yoghurt are of great importance for the normalization of the autochthonous flora and the consequent increase of the bodyís natural defence against infections diseases and putrefactive and fermentative processes (Biachi-salvadori,1986) probiotics are live microbial supplements, which beneficially affect the host by improving its intestinal microbial balance (Fuller,1992 and Tiina Mattila, et.al 2003). The therabeutic value of lactobacills acidophilus has been stablished for many diseases and disorders of the digestive tract (Danielson, et.al,1989). Probiotic bacteria produce B-galactosidase which is beneficial for people with lactose intolerance. The normal yoghurt cultures, lactobacillus, delbrueckii ssp. bulgaricus and streptococcus thermophilus, produce B-galactosidase in yoghurt but these bacteria cannot servive and grow in the intestinal tract due to their low bile salt tolerance. In contrast, probiotic bacteria such as Lb. acidophilus and Bifidobacterium bifidum can survive and grow in the intestinal tract and produce B-galactosidase in the presence of bile salt (Noh & Gilliland 1993). In addition, the used of certain Lactic acid bacteria to assimilate cholesterol invitro (Gilliland and Kim,1984; Lin et.al.,1985; Gilliland & walker,1990, Sanders Ellen., 1993, and Pereira, 2002) or to lower blood cholesterol invivo (Danielson et.al.,1989 and Portugal et.al., 2006) Thus,encapsulated probiotic bacteria can be used in many fermented dairy products such as Yoghurt, Cheese, Cream and frozen dairy desserts (Wunwisa et.al 2003) . Numerous studies, however, have demonstrated that *Lactobacillus acidophilus, Bifidobacteria* bifidum, and Lactobacillus bulgaricus bacteria lower cholesterol in a significant fashion. ( Sanders Ellen 1993, Taranto et.al; 1998, Pigeon et.al; 2002, Kimoto et al; 2002, and Tiina Mattila et.al., 2003).

## MATERIALS AND METHODS

**A-In vitro:-**

**Probiotic strains:**

*L. acidophillus* N10, *L. bulgaricus N5, L.casei* B20, *Bifidobacterium* spp420, L. helveticus H42, obtained from Danisco cultor Niebull GmbH., *Str.thermophllus* R, *L.plantarium* C12, Str. CremorisF50, *L. delbrueckii spp* 12 obtained from American type culture collection (Rockville, MD.USA) while *L. acidophillus* N10, *Bif. Longum K69, Bif.therm-bifidum* 9, Bif.spp 815, *Str. thermophillus* S1, *Str. thermophillus* S2 were obtained from Agricultural Research Service- peoria, illinois Midwest Area.

Skim Milk Powder: DIFCO 500 g - Casein soluble: NOURESHARK Co. Egypt-

Chlesterol Powder: PROLABO 250 g- MRS broth and GM17 broth Media from OXIOD Co.- cholic acid, and coconut oil from sigma Co. USA.

Powderd Milk 500 g from MERK Co. Germany

**Maintenance of probiotic strains:** were adopted according to ( Taranto, et al, 1998; Kimoto, et al, 2002 and Pigeon, et.al, 2002)

**Analysis of cholestrol and triglycerides in broth medium :** were performed according to (Rudel and Morris, 1973 and Kimoto, et al 2002)

**Yoghurt processing:** according to (Abdel Twab 1967)

**B- *In vivo*:-**

**Animals:**

A total number of 40 male albino rats weighing (120±30 g B.wt) were used. The animals were brought from our breeding center of NODCAR and kept under strictly hygienic conditions. They were put on a stander basal diet and allowed free access to drinking water.

**Experimental Technique:-**

***Induction of hyperlipidemia*:**

Hyperlipidemia in rats was done according to the method of Grone et.al 1989) by feeding the animal high fat (20% coconut oil)/ cholesterol (1%) for 2 month. The high fat diet contained cholic acid (0.5%) to enhance the central absorption of lipids. The occurrence of hyperlipidemia was determined by measuring of lipid profile for the animals.

hyperlipidemic rats were classified into five equal groups each comprise 6 rats and treated for 2 weeks by 3 different starter of strains as follow:

1. Positive control group{ (+)ve C}.
2. starter (B) = SB treated group (1 $x10^7$cfu/ml/ rat/day).= yoghurt B
3. starter (H) = SH treated group (1 $x10^7$cfu/ml/ rat/day).= yogurt H
4. starter (E) = SE treated group (1 $x10^7$cfu/ml/ rat/day).= yoghurt E

At the end of the treatment schedule, blood samples were taken from each rat and they were sacrificed, aorta tissues were removed and subjected

to histopathological examinations as described by Bancroft and Stevens (1977).

Separated serum were processed for the bio-chemical analysis, T-Ch, HDL-Ch, TG were done according to the method of commercial available kits LDL-Ch was calculated by the formula of Friedwald, et al (1972) total fatty acids was determined by GC chromatography according the method of Xu, et al., (1993).

**Statistical analysis** were done using the means of each group and compared for significant differences using Student's t-test.

## RESULTS

### A- in vitro study:-

Results in table (1) showed that, the highest strains of lactic acid bacteria for removal cholesterol (T-ch) and triglycerids (TG) were 20% of (T-ch ) and 19.2% of (TG) for *L.bulgaricus* N5, 19.6% of (T-ch ) and 19% of (TG) for *Bif.Longum K69*, 19.4% of (T-ch) and 20% of (TG ) for *L.acidophillus* N10, 18.9% of (T-ch) and 16.2% of (TG) for *Str.thermophillus* S1, 15.3% of (T-ch) and 15% of ( TG) for L.casei B20, 12.5% of (T-ch) and 13.6 % of (TG) for *Bif.therm-bifidum* 9 and 12.5% of (Tch) and 13% of (TG) for *L.helveticus* H42 compared with another strains which removed (T-ch) and (TG ) smallar than that by above mentioned strains . On *the other hand, there are no effect of L.plantaium C12 and Bif.Spp* 815 on cholesterol and triglycerides.

Results in table (2) revealed that, the best reduction of (T-ch) and (TG) were group B which gaves 20.3% µg/ml of (T-ch) reduction and 25.2% Ug/ml of (TG) reduction, while group H gaves 19.8% µg/ml of (T-ch ) and 18.0% µg/ml of (TG ) reduction and group E gaves 18.8% µg/ml (T-ch ) and 16.8 µg/ml of (TG) reduction compared with blank resp.

**Table (1) Effect of probiotic strains on cholesterol (T-ch ) and Triglycerides (TG) in broth media after 24 h of incubation period.**

| Strains of L.A.B / Removal % µg/ml | $10^7$ c.f.u | | $10^6$ c.f.u | | $10^5$ c.f.u | |
|---|---|---|---|---|---|---|
| | T-ch | TG | T-ch | TG | T-ch | TG |
| 1- L.acidophillus N11 | 10.5 | 10.0 | 9.3 | 9.2 | 8.4 | 8.0 |
| 2- L.casei B20 | 15.3 | 15.0 | 15.2 | 14.0 | 14.2 | 14.3 |
| 3- L.acidophillus N10 | 19.4 | 20.0 | 16.3 | 18.2 | 16.0 | 18.0 |
| 4- L.bulgaricus N5 | 20.0 | 19.2 | 19.6 | 18.4 | 17.3 | 17.0 |
| 5- L.plantarium C12 | 0.0 | 1.2 | 0.0 | 1.0 | 0.0 | 0.0 |
| 6- L. helveticus H42 | 12.5 | 13.0 | 12.3 | 12.5 | 12.0 | 11.0 |
| 7- Str. Thermophllus R | 3.9 | 2.5 | 3.2 | 2.2 | 2.0 | 0.0 |
| 8- Str. CremorisF50 | 5.6 | 4.4 | 5.0 | 4.0 | 4.8 | 3.2 |
| 9- Bifidobacterium spp | 8.2 | 6.0 | 8.0 | 6.0 | 7.3 | 5.8 |
| 10- Bif. Longum K69 | 19.6 | 19.0 | 19.2 | 18.5 | 19.0 | 18.2 |
| 11- Bif.therm-bifidum 9 | 12.5 | 13.6 | 12.0 | 12.2 | 11.3 | 12.0 |
| 12- Bif.spp 815 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| 13- Str. thermophillus S1 | 18.9 | 16.2 | 18.6 | 16.0 | 18.0 | 15.0 |
| 14- Str. thermophillus S2 | 2.6 | 8.6 | 2.2 | 8.0 | 2.0 | 7.1 |
| 15- L. delbrueckii spp12 | 4.6 | 3.2 | 3.6 | 2.9 | 3.2 | 2.0 |

The mechnism by which lactic acid bacteria to emove cholesterol from laboratory media has been studied and reported by (Klaver & Van der Meer 1993 and Kimoto, et al., 2002) that cholesterol removal by some lactobacilli is only due to a disruption of the cholesterol micelles caused by the deconjugation and precipitation of cholesterol with the free bile salts as the pH of the media dropped by acid production duting the growth. These results are agreement with numerous studies (Gilliland et al., 1984, Lin et al.,1989, Gilliland &Walker, 1990, Fuller, 1992, Gopal, et al., 1996, Kimoto, et al., 2002 and Pigeon, et al., 2002).

**Table(2) Effect of some suggested probiotic strains ( starter yoghurt) on cholesterol and triglycerides in yoghurt after 24 h of incubation.**

| Suggested group Or (Yoghurt ) | Removal % µg/ml | |
|---|---|---|
| | (T-ch) | (TG) |
| A | 1.5 | 1.0 |
| B * | 20.3 | 25.2 |
| C | 13.1 | 12.2 |
| D | 7.5 | 5.2 |
| E * | 18.8 | 16.8 |
| F | 3.2 | 2.1 |
| G | 6.2 | 4.1 |
| H * | 19.5 | 18.0 |
| I | 2.1 | 2.2 |
| J | 1.2 | 3.1 |
| Blank | 0.0 | 0.0 |

* Starters were inoculated by 1%
* Blank= normal starter of lactic acid bacteria
*Starter B= *L.acidophillus N10, Str.cremoris F 99 and Bif.ther-bifidum99*
*Starter E= *L.acidophillus N10, Str.cremoris F50, L.bulgaricus N5 and Bif-longum K69*
*Starter H= *L.acidophillus N10, L.bulgaricus N5 and Str.thermo*phillusS1

**B-*in vivo* study:-**

**Biochemical results:**

As depicted in table (3) and fig.(1), oral administration of high fat diet containing cholesterol and cholic acid caused a significant increase (P<0.05) of total serum cholesterol, LDL-Ch, and TG as well as a significant reduction (P<0.05) in the level of serum HDL-Ch. Furthermore a marked increase (P<0.05) in the level of serum TFA, SFA & UFA were recorded in (+)ve C group, while the ratio of UFA/ SFA was significantly (P<0.05) decreased (40%) in respect to the value recorded in (-)ve C group.

**Table(3) &Fig(1):Effect of hyperlipidemic induction on the level of serum lipid profile (mg/dL) and fatty acids (mg/dL) for 45 days.**

| Parameter | (-)ve C | (+)ve C | | % Change between (-)ve C & (+)ve C(B) |
|---|---|---|---|---|
| | | Before treatment(B) | After treatment (A) | |
| T-Ch | 75.17 ± 1.19 | 115.50 ± 4.50* | 109.67 ± 6.03* | 54% |
| TG | 101.50 ± 3.96 | 166.67 ± 6.17* | 158.50 ± 6.71* | 64% |
| LDL-Ch | 22.17 ± 1.19 | 59.17 ± 3.39* | 55.76 ± 2.91* | 167% |
| HDL-Ch | 32.67 ± 2.47 | 23.67 ± 1.45* | 24.33 ± 1.35* | (-)26% |
| TFA | 295.83 ± 7.51 | 522.00 ± 27.58* | 513.67±29.35* | 76% |
| SFA | 125.00 ± 4.19 | 287.33 ± 13.16* | 279.17±11.14* | 130% |
| UFA | 170.83 ± 3.58 | 234.67±17.41* | 228.67±7.44* | 37% |
| Ratio of UFA/SFA | 1.37 ± 5.44 | 1.32 ± 17.44 | 1.32 ± 2.44 | (-)4% |
| | | | | |

Data are expressed as mean values of 6 rats ± SE.
Significance difference from (-) ve Control group: *P<0.05.

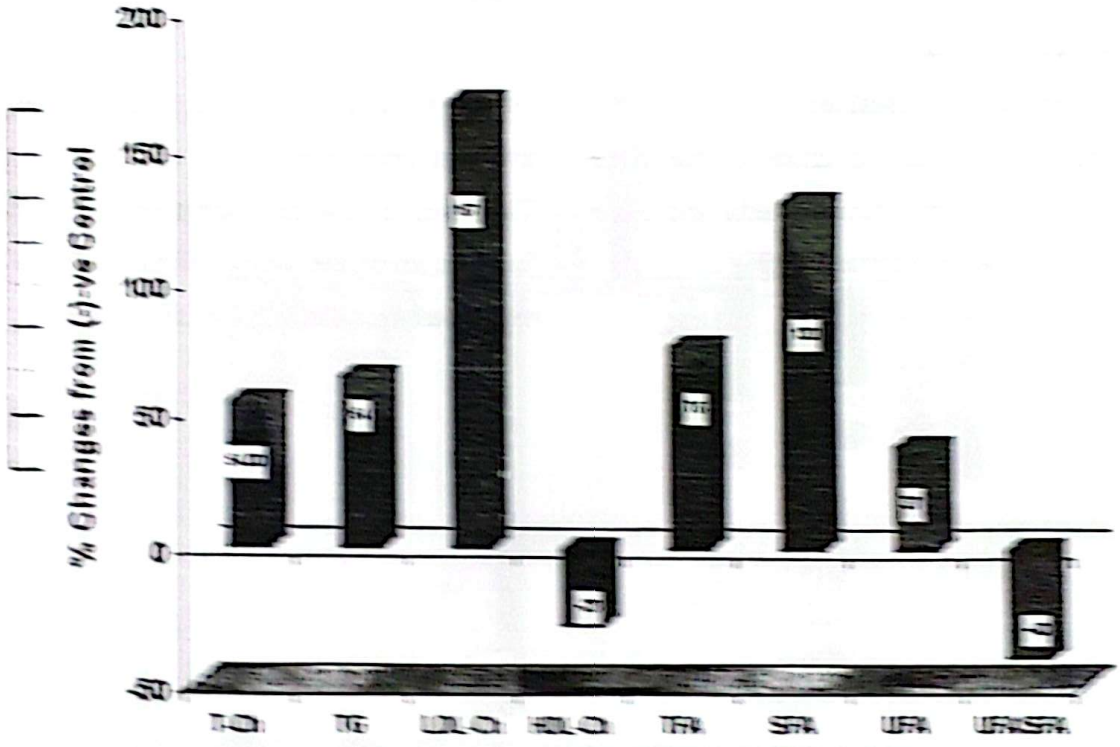



Fig. (1): %Changes of lipid profile and fatty acids from (-)ve Control group.

To evaluate the treatment effect of the tested starters, the data recorded in table (4) and Fig. (2), showed that all animals treated with the tested strains experienced similar significant reduction ($P<0.05$) in serum T-Ch, LDL-Ch and TG as well as significant increment ($P<0.05$) in the level of HDL-Ch in parallel to the values of (+)ve C group. Furthermore the level of serum TFA, SFA & UFA were decreased significantly ($P<0.05$) regarding to the value of (+)ve C group. The data also showed that rats treated with group (H) have the more pronounced effect in reducing the level of TFA in respect to the values of other tested starters. In addition the ratio of UFA/SFA was increased significantly ($P<0.05$) in group of rats treated only with group (H) in parallel to values of (+)ve C group .

*Histopathological study*:

**Group of rats kept as control:**

The normal histological structure of the aorta formed of tunica intima, tunica media and outer tunica adventitia were recorded in (Fig a)

**Group of experimental hyperlipidemic rats:**

Haemorrhage was detected in the perivascular tissue ( Fig. b1 ), with oedema in the tunica adventitia, vacuolation in the cells of the media and desquamation in the endothelium of the tunica intima ( Fig. b2 ).

**Group of hyperlipidemic rats treated by H group of probiotics :**

The tunice intima and media were intact and the normal histological structure of tunica adventitia were recordes in ( Fig. C ).

**Group of hyperlipidemic rats treated by B group of probiotics :**

Some of the endothelial cells lining the tunica intima were desquamated while other showed swelling ( Fig. D ).

**Group of hyperlipidemic rats treated by E group of probiotics:**

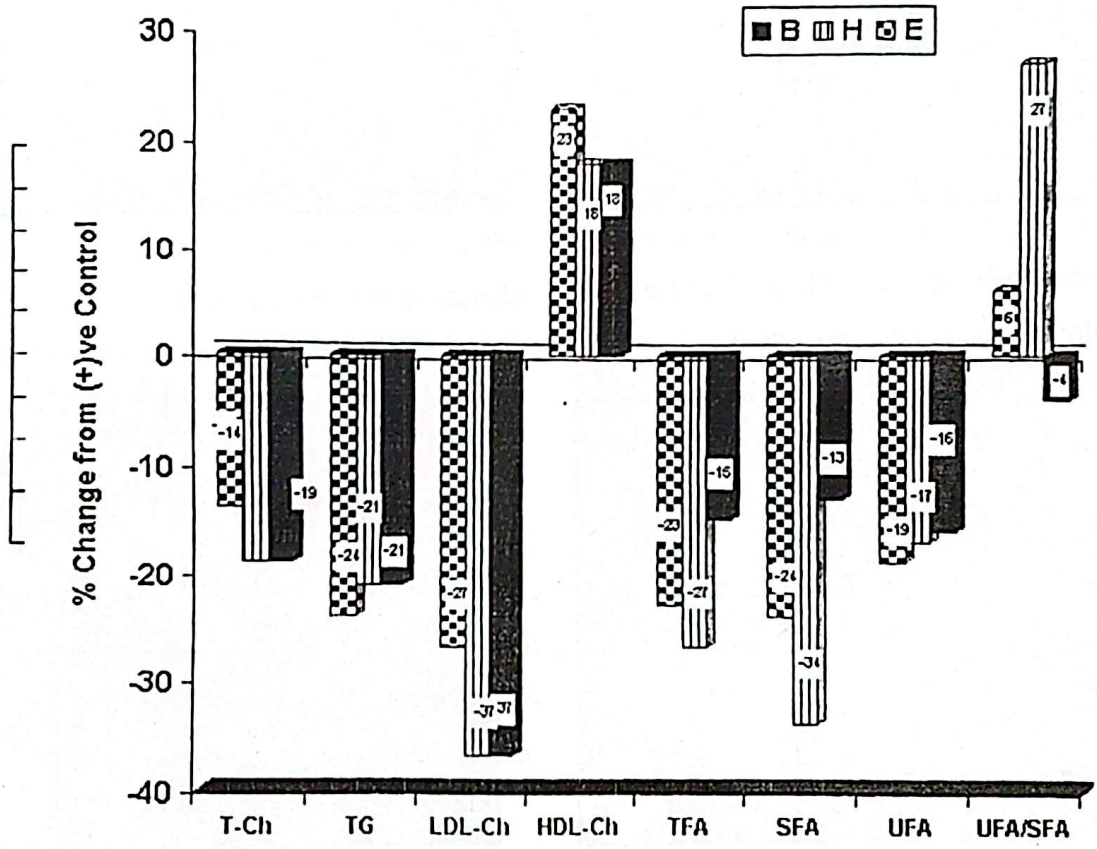
There was diffuse desquamation in the endothelial cells lining the tunica intima while the tunica media had vacuolation ( Fig. E ).

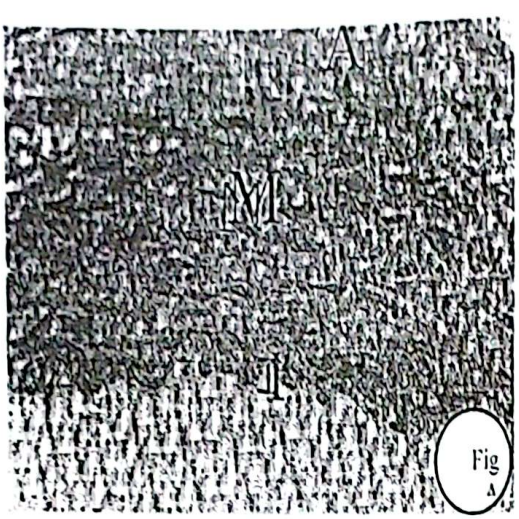
**Table(4) &Fig(2):The effect of different treatments on the levels of serum lipid profile and fatty acids(mg/dL) against hyperlipidemic rats after 15 days from treatment.**

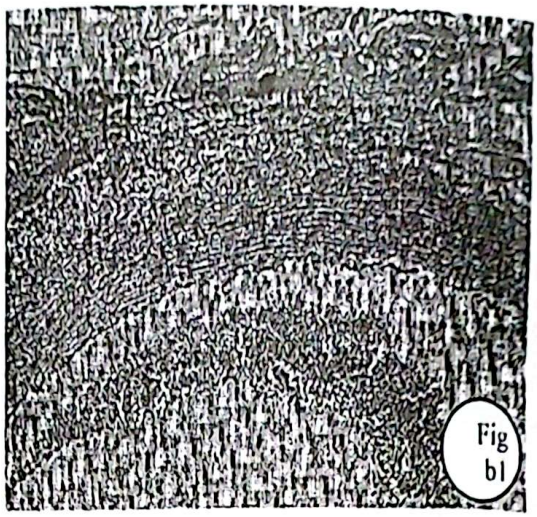
| Paramter | (+)ve C | B | H | E |
|---|---|---|---|---|
| T-Ch | 109.67 ± 6.03 | 88.50 ± 2.54* | 89.17 ± 1.51* | 94.67 ± 3.35* |
| TG | 158.50 ± 6.71 | 124.67 ± 8.89* | 125.50 ± 7.18* | 121.00 ± 4.55* |
| LDL-Ch | 55.76 ± 2.90 | 34.9 ± 1.24* | 35.24 ± 0.70* | 40.47 ± 1.68* |
| HDL-Ch | 24.33 ± 1.33 | 28.67 ± 2.17* | 28.83 ± 1.94* | 30.00 ± 3.16* |
| TFA | 513.67±29.35 | 435.17 ± 18.19* | 373.33 ±18.53* | 397.67 ± 17.61* |
| SFA | 279.17±11.14 | 243.67 ± 11.46* | 183.33 ± 10.73* | 213.00 ± 9.03* |
| UFA | 228.67±7.44 | 191.50 ± 8.96* | 190.00 ± 4.58* | 184.67 ± 7.33* |
| Ratio of UFA/SFA | 0.82 ± 8.44 | 0.79 ±10.22 | 1.04 ± 7.00* | 0.87± 6.44 |

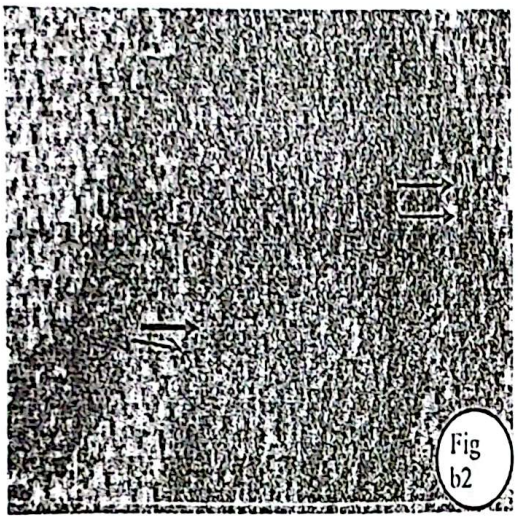
Data are expressed as mean values of 6 rats ± SE

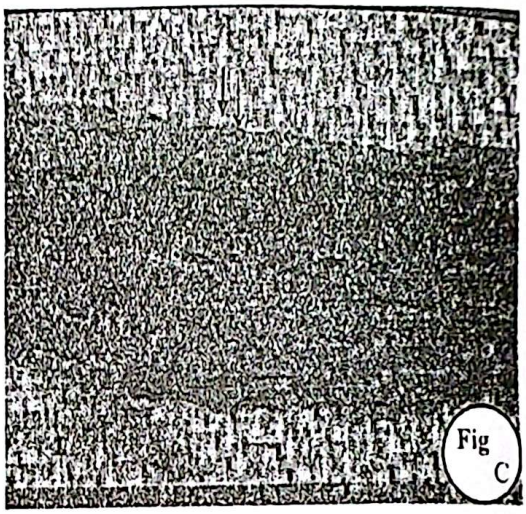
Significance difference from (+)ve Control group: *P<0.05.

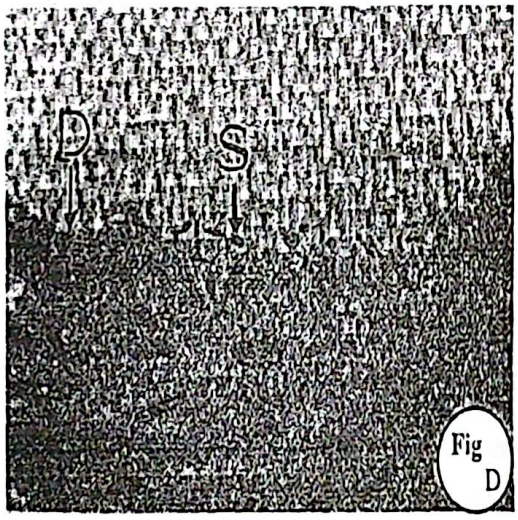




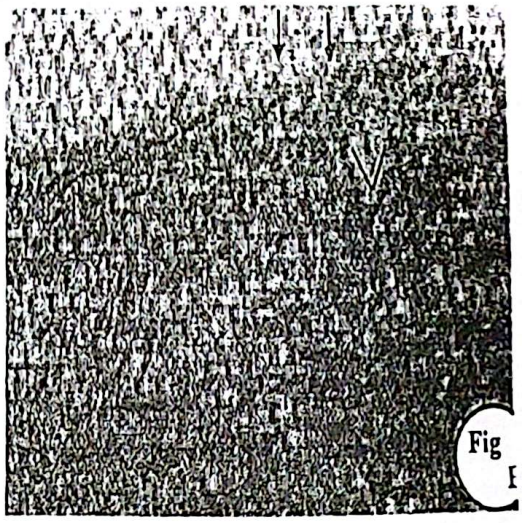



**Fig (a)**: Aorta of rat in control group showing the histological structure of the tunica intima (I), media (M) and adventitia (A). H & E X160.

**Fig. (b1.)**: Aorta of hyperlipidemic rat showing perivascular

Haemorrhages (H). H & E X 40

**Fig. (b2)**: Aorta of hyperlipidernic rat showing vacuolation in the cell of Tunica media ( arrow ) and desquamation of the endotheliun of the endotheliun of tunica intima ( double arrow ). H & E X160

**Fig. (C)**: Aorta of hyperlipidemic rat by Starter H showing intact lining endothelium of the tunica intima ( arrow) and normal structure of aorta.

H & E X160

**Fig. (D)** : Aorta of hyperlipidemic rat treated by Starter B showing desquamation of the endothelial cells lining the intima ( D ) with swelling in other ( S ).H & E X160

**Fig. (E)** : Aorta of hyperlipidemic rat treated by Starter E showing desquamation of the endothelial cells lining the intima ( arrow ) with vacuolation in the media ( V ). H & E X 160 .

## DISCUSSION

The elevated levels of T-ch, TG, LDH-ch as well as the decreasing level of HDL-ch beside the histopathological changes in aorta tissues recorded in the results indicated the strong correlation between hypercholesterol-emia and incident of atherosclerosis (Stary et al., 1992).

The result of present study demonstrated that, as compared to different ten selected starters for lowering T-h and TG, three chosen group of probiotics for yoghurt as (B,H, and E) were more effective in improving serum lipid in experimental hyperholesterolemic rats. In comparison with the control, all the data obtained from the 3 selected starters experienced some what similar significant reduction in the levels of serum lipid parameters.

The results indicated that 3 fermented milk as (yoghurt) were lowered serum total cholesterol concentrations in rats agree with data from others studies involving various milk product containing selected strains of lactic acid bacteria (Akalin etal.,1997 and Beena & Prasad, 1997). Reduction of T-ch, TG were observed with selected strain including *probiotic bacteria (L.acidophilus, L.bulgaricus, Str. Cremoris, Str.thermophillus, Bifidobacterum longum, and Bif.ther-bifidum)* decrease in T-ch, LDL-ch with a simultaneous increase in HDL-ch. The observation in this study agree with the finding of Taranto et al., (1998) and Hashimoto et al., (1999).

The mechanisms of serum lipid improvement have been suggested from invitro and invivo studies. In vitro experiments demonstrated that intestinal lactic acid bacteria have the capacity to assimilate and bind cholesterol as well as bile acids with the bacterial cells (Hosono & Tono-oca

1995 and Kimoto et al., 2002). From these results, it is conceivable that serum total cholesterol is reduced by inhibiting absorption in the intestine as a result of the assimilating and binding of cholesterol as well as bile acids by the lactic acids bacteria. On other hand serum lipid improvement seen to be related to the hydrolyase activity of the bile salts of the bacterial cells that deconjugate about half the bile of the conjugated bile salts that are excreted into the small intestine which would increase the fecal excretion of deconjugated bile salts. To maintain bile salts homeostasis, the bile acid have to be newly synthesized in the liver thus increasing the demand for cholesterol as precursor for bile acids and this leading to lower the level of cholesterol concentration (Taranto et al., 1998 and Pereira & Gibson., 2002). Surviving passage through gastrointestinal tract is believed to be important for proboitics strains as *L.acidophilus, L. bulgaricus, Str. Cremoris, Str. thermophillus, Bifidobacterum longum, and Bif.ther-bifidum* may be due to bind with cholesterol and bile acids as well as by suppressing bile acid resorption by deconjugation. (Salminen and vonWright, 1993 and Xiao et al., 2003).

The significant reduction of serum total fatty acids observed in this study with the 3 selected starters, showed that group H ($S_H$) had the most powerful reduction in order that $S_H > S_E > S_B$. Only $S_H$ could restore ratio of UFA/SFA by increasing the level of UFA and decreasing the level of SFA in corresponding to the values recorded in control group.

It was reported by the study of Kimoto et al., (2002) that some bacteria can remove cholesterol from media both by binding cholesterol to dead cells on the cell surface and by uptake of cholesterol into the living cells. As it was known that, the lipid of the gram positive bacteria found predominately in the membrane, suggesting that cholesterol was incorporated into the cellular membrane of the bacteria and may have altered the fatty acids composition. The author reported an increase in the level of UFA and decrease in the level of SFA. These result were in good keeping with the present study that showed an increase in the ratio of UFA/SFA of serum of rats administrated group H suggesting that the increasing of UFA on the surface of probiotic strains bacteria may reflect an increase in the serum UFA of rats fed on group H.

The association between nutrition and hyperlipidemia or the production of premature atherosclerosis is mainly due to the effect of nutrients on serum lipids, lipoprotein (LDL-Ch, HDL-Ch) that are considered the common risks of atherosclerosis. So cholesterol and saturated fatty acids intake, strongly negatively affected plasma total cholesterol and LDL-Ch while mono and poly unsaturated fatty acids are generally regarded as

beneficial (Haban, 2000 and Suzan, 2003). Thus nutrition with group H was considered the best nutriment as it is not only decrease the level of serum cholesterol but also increase the level of UFA.

In connection with histopathological changes, the moderate protective effect on aortic atherosclerosis associated with the 3 selected yoghurt made by above mentioed groups (B, H, and E) and treatment specially with group SH, is secondary to their attenuation of induced hypercholesterolemia and reflects their hypocholesterolemic effect.

## CONCLUSIONS

The present study demonstrated that, as compared with of some probiotic strains traditional yogurt fermented with ordinary lactic acid bacteria, as *L.acidophilus, Str. Cremoris, Bifidobacterium longum, and Bif.ther-bifidum* were effective in improving serum lipid in rats which possess a high tolerance to gastric and bile acids and show assimilating and binding of cholesterol, a strong bile salt hydrolase activity, strong survive passage through the gastrointestinal tract. Also it considered benefit nutriment as it provide diet rich in UFA and finally their potent ability to regain the normal architecture of aorta tissues of hypercholesterolemic rats to be as observed in normal rats.

strains through the yoghurt.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## REFERENCES


Abdel-tawab, G. Eldeen (1967): Principles of Dairy industry . Daar.Elmaref-Egypt.

Akalin, A.S.; Gonc, S and Duzel, S .(1997): Influence of yoghurt and acidophilus yoghurt on serum cholesterol in Mice. J.Dairy Sci.; 80:2721-2725.

Beena, A; and Prasad.V, (1997): Effect of yoghurt and bifidus yoghurt fortified with skim milk powder, Condensed whey and lactose-hydrolysed condensed whey on serum Cholesterol and triacylglycerol levels in rats. J. Dairy Res.64:453-457.

Biachi-salvadori, B.(1986): Intestinal microflora: the role of yoghurt in The equilibrium of the gut ecosystem int.j. Immunother. suppl.11, 9-18.

Brashears, M.M., Gilliland, S.E and Buck, L.M.(1998): Bile salt deconjugation and cholesterol removal from media by Lactobacillus casei. J.Dairy Sci.81: 2103-2110.

Buck, L.M., and Gilliland, S.E. (1994): Comparisons of freshly isolated strains of to assimilate cholesterol during growth J. of Dairy Sci.72,2885-2899.

Bancrofet, J.D and Stevens, A. (1977):"Theory and prac-

tice of histological technique " Churchil living-stone Edinburgh- london and New York. p. 89.

Danielson A.D.; Peo.E. R.J.R.; Shahani, K.M.; Lwis.A.J., Whalen. P.J.; and Amer, A.M. (1989): Anticholesteremic property of lactobacillus Acidophilus yoghurt fed to moture boars. J. Anim. Sci. 67, 966-974.

De Man, J.C., Rogosa, M and Sharpe, M.E.(1960): Amedium for the cultivation of lactobacilli J. Appl. Bacteriolg. 23:130-135.

Friedwald, W.T., Levy, R.I, and Fredrickson, D.S. (1972): Estimation of the concentration of LDL- C in plasma without use of preparative ultracentrifuge. Clin. Chem. p.499.

Fuller, R (1992): Probiotics: the scientific basis London :Chapman &Hall. Copyright 1993 by Academic pPress.

Gopal, A.N.P.Shah, and Roginski. (1996): Bile tolerance, taurocholate deconjugation and cholesterol . Removal by *Lactobacillus acidophilus and Bifidobacterium* Spp. Milchwiss. 51: 619-623.

Gilliland, S.E., and Kim, H.S. (1984): Effect of viable starter culture bacteria in Yoghurt on lactose utilization in humans J.of dairy Sci 67, 1-6 .

Gilliland, S.E., and Walker, D.K (1990): Factors to consider when selecting a culture of *lactobacillus acidophillus* as adietary adjunct to produce a hypocholesterolemic effect in humans J.of Dairy Sci. 73, 905-911.

Grone, H.J.; Walli, A.K.; Grone, J.T.; Seidel, D. and Helmchen, U. (1989): Induction of glometulsclerosis by dietary lipid. A functional andmorphological study in rats. Lab. Invest .60: 433-446.

Grunewald, K.K. (1982):Serum Cholesterol Level in Rats Fed Skim milk fermented by Lactobacillus acidophilus. J. Food Sci. 47: 2078-2079.

Haban, P. (2000): Supp; imentation its long-chain n-3 fatty acid in non-insulin-dependent diabetes mellitus (NEDM) patients leads to the lowering of oleic serum phospholipids. Eur. J. Nutr.39: 201-206.

Hashimoto, H., K. Yamazaki, F.He, M.Kawase, M. Hosoda, and Hosona. (1999): Hypocholesterolemic effects of lactobacillus casei subsp. Casei TMC 0409 strain observed in the rats fed cholesterol contamined diets.Anim.Sci. J.72: 90-97.

Hosono, A., Tanako, T. (1995): Binding of cholesterol with lactic acid bacterial cells Milchwiss, Vol 50 pp 556-560.

Kimoto, H; Ohmomo, S and Okamoto, T. (2002): Cholesterol Removal from Media by *Lactococci.*

J. Dairy Sci.85:3182-3188.

Klaver, F.A.M., and Van der Meer, R. (1993): The assumed assimilation of cholesterol by *lactobacilli and Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59:1120-1124.

Lin, S.Y., Ayres, J.W., Winkler,W., Jr., and sandine,W.E. (1985): Lactobacillus effects on cholesterol : in Vitro and in vivo results. J. Dairy Sci. 72, 2885-2899.

Mabuchi, H. (1999): Primary and secondary prevention of atherosclerotic disease By lipid-lowering therapies. Nippon Rinsho 57:2807-2814.

Moore, S. (1996): Blood vessels and lymphatics. In: anddersons pathology I. Damjanov and J. Linder, eds). 10th ed. Missouri:

Mosby Year Book, Inc. Noh, D.O. and Gilliland, S.E. (1993): Influence of bile on cellular integrity and Beta-galactosidase of *Lactobacillus Acidophilus* . J. of Dairy Sci,76 (5) 1253- 1259.

Oxoide Manual. (1991): The oxoid manual of culture Me-

dedia and Other Laboratory Services

ereira D.I.A and Gibson G.R., (2002): Effect of Consumption of Probiotics and Prebiotics on Serum Lipid Levels in Humans. Critical Revieews in Bichem.and Molecular Biol. 37(4)259-281.

igeon R.M, Cuesta E.P, and Gilliland S.E.,(2002): Binding of free Bile Acids by Cells of Yoghurt Starter Culture Bacteria J. Dairy Sci.85: 2705-2710.

ortugal, L.R.; Goncalves, G.L.; Fernandes, L.R.; Silva, H.P.; Arantes, R.M.; Nicoli, G.R.; Vieira, L.Q and Alvarez-Leite, J.I . (2006): Effect of lactobacillus delbrueckii on cholesterol metabolism in germ- Free mice and on atherogenesis in apolipoprotein E knock-out mice Braz. J. Med. Biol. Res. Vol. 39 (5):629-635.

Rudel, L. L., and. Morris. M. D. (1973): Determination of cholesterol using o-phthalaldehyde. J. Lipid Res. 14: 364-366.

Salminen, S., and Von Wright. A.(1993): Lactic acid bacteria. Marcel Dekker, Inc., New Yorker, NY.

Sanders Ellen M, (1993): Effect of Cosumtion of Lactic Cultures on Human Health Advances in Food andNutrition Res.Vol 37:67-131.

Stary H.C.; Blankenhorn, D.H.; Chandler, A.B.(1992): A defination of the intimia of human arteries and of its atherosclerosis-Prone region. Atherosclerosis 12 (1): 120-134.

St-Onge, M.P., Farnworth. E.R. and Jones P. J. H. (2002): Consumption of fermented and non fermented dairy products : Effects on cholesterol concentration and metabolism. Am. J. Clin. Nutr. 71:674- 681.

Suzan F.I. El-Sisi. (2003): The role of combined treatment of B-vitamins and Omega-3 Fatty acids In experimental atherosclerosis. J. of genetic Eng. & Biotechnol. (NRC)1, No., 2PP, 423-438.

Taranto. M. P.; Medici, M, Perdigon.G.; Ruiz A.P. Holgado, and Valdez G. F. (1998): Evidence for hypocholesterolemic effect of *Lactobacillusreuteri in* hypercholesterolemic mice. J. Dairy Sci. 81: 2336-2340.

Tiina Mattila, Sandholm and Maria Saarela, (2003): Functional Dairy Products.Woodhead publishing Ltd. And CRC press LLC -Cambridge England.): Bile tolerance, taurocholate deconjugation, and binding of cholesterol by lactobacillus gasseri strains J. Dairy Sci. 82:243-248.

Wunwisa .K; Bhesh B, and Hilton D, (2003): Evaluation of encapsulation techniques of probiotic for yoghurt International Dairy Journal 13: 3-13.

Xiao J. Z., Kondo S., Takahashi N, Miyaji K., Oshida K.,Hiramatsu A., Lwatsu (2003):Effect of Milk Products Fermented by *Bifidobacterium* longum on Blood Lipid in Rats and Healthy Adult Male Volunteers. J. Dairy Sci.86:2452- 2461.

Xu, Z.C.; Li, X.Y.; Jiang. J.X.; Yin, X.; Shen. S.Y.; Yang. J.Z. (1994): Determination of free fatty acid in the plasma of patients with malignant Hematopoietic diseases by gas chromatography- Sepu: 12 (4) 268-270.

Yamamoto A., Horibe H., Mabuchi H., Kita T., Matsuzawa Y., Saito Y, Nakaya N.,

Fujioka T., Tenba H., Kawaguchi A., Nakamura H., and Goto Y. (1999): Analysis of serum lipid levels in Japanese men and women according to Body mass index. Increase in risk of atherosclerosis in postmenopausal. Women. Research group on serum lipid survey (1999): in Japan. Atherosclerosis 143: 55-73.